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Sublethal Effects of Number 2 Fuel Oil

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LOBSTER BEHAVIOR AND CHEMORECEPTION: SUBLETHAL EFFECTS OF NUMBER 2 FUEL OIL

by

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16. ABSTRACT

This research has demonstrated behavioral abnormalities and inappropriate responses in lobsters exposed to levels of 0.1-1.0 parts per million (ppm) of oil in water. Such abnormalities can lead to lack of feeding and population decline; they occur at exposure levels below those that cause obvious loss of equilibrium and coorination,and which eventually lead to death of the organism.

In an attempt to explain the mechanisms by which the behavior deteriorates, two topics were examined: interference with normal smell and taste, and change of motivation. A combination of neurophysiological and behavioral experiments on chemoreception were designed to investigate these topics.

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FOREWORD

The Narragansett Environmental Research Laboratory of the U.S. Environmental Protection Agency is conducting research to develop a firm, factual basis for regulatory decisions designed to protect man and his environment from damaging effects of pollutants in marine waters. Information needed in such decisions includes:

- Physical and chemical fate and transformations of pollutants in marine systems
- Responses of marine organisms to pollutant stress
- The resultant effects of different organism and community responses on the integrity of ecosystems and biogeochemical processes

This report describes the sublethal responses of the American lobster, Homarus americanus, to the presence of oil in their environment and attempts to relate those responses to the probable effects of releases to the environment.

Tudor T. Davies
Director

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ABSTRACT

The feeding behavior of adult lobsters was subjected to careful multidisciplinary analysis for the sublethal effects of No. 2 fuel oil. Six lobsters were exposed to a water accommodated fraction (WAF) of this oil at 0.1, 0.3, and 1.5 ppm for 5 days. A period of 5 days before the WAF was added served as internal control, and each 15-day experiment concluded with a 5-day recovery period. For external control, a group of 4 lobsters was observed under identical conditions but without oil exposure. All animals were acclimated in their test tanks for at least one week prior to the beginning of observations. WAF exposure levels were determined by chemical analyses (infrared and ultraviolet spectroscopy and gas chromatography) of water samples from lobster tanks. Feeding behavior was measured twice daily using the duration of five specific phases of feeding behavior, namely ALERT, WAIT, SEARCH, HIT, AND EAT. General behavior and postures were also described in discrete behavior units prior to and during food search. Experiments were carried out from March through November under ambient light regimes and in ambient water temperatures ranging from 8-23° C. Neurophysiological measurements were performed on the antennular chemoreceptors to determine if malfunctioning chemoreception was (one of) the cause(s) of behavioral deficits caused by oil exposure.

Chemical analyses showed that the intended exposure levels were approximately achieved and maintained for the 5 exposure days. Control tanks contained 0.03-0.08 ppm recovered lipids but no detectable petroleum hydrocarbons. Exposures of 0.1 ppm only rarely caused measurable effects on feeding behavior. At 1.5 ppm serious neuromuscular defects became apparent after a few hours of exposure, lasting throughout the 5-day recovery period in clean water after only a 30 hr exposure period. Five out of 6 such animals could hardly walk and twitched in cramped postures, responding poorly or inappropriately to chemical food stimuli. The 0.3 ppm exposed group was the most interesting for sublethal effects. Most animals showed delays in feeding responses after 6 hours or after 24 hours of exposure and usually required one day for recovery. Behavioral changes were apparent in both the occurence and duration of feeding behavior phases. Some animals failed to ALERT to food, others SEARCHED but did not HIT, etc., with the result that most lobsters missed several feedings during the exposure period.

Neurophysiological recordings of primary chemoreceptors on the antennules showed that non-dosed control animals received No. 2 fuel oil (WAF) as a chemical stimulus, and that this petroleum fraction can cause a change in the normal response pattern to food odors. Animals from the 0.3 ppm exposed group showed abnormal spontaneous activity patterns as well as abnormal response patterns to food odors. Their receptors showed weaker responses to oil stimuli than did receptors from control groups.

Despite the observed effects on antennular chemoreception we currently do not believe impaired chemoreception to be the (major) cause for delays and failures observed in feeding behavior in the 0.3 ppm WAF group because animals without antennules show similar behavioral deficits. Moreover, phases of feeding behavior under clear chemosensory control, such as ALERT and SEARCH, are not affected more than other phases, such as WAIT. This would indicate a change in motivational state. It is possible that certain petroleum fractions cause general neurotoxic effects; at 0.3 ppm oil may affect sensory modalities while at 1.5 ppm, the motor nerves and centers may be affected as well.

This report was submitted in fulfillment of Grant No. R-803833 by Boston University under the sponsorship of the Environmental Protection Agency. This report covers the period 1 November 1975 to 31 March 1979.

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SECTION 1

INTRODUCTION

Due to increased oil transportation and off-shore drilling, measuring the effects of oil pollution on marine organisms that live in affected areas has become an important area of research. Various petroleum hydrocarbons at high concentrations have been shown to be lethal to many marine species. At lower, sublethal concentrations oil may still interfere with certain life processes which over time may decrease populations without directly killing individuals. Yet sublethal pollution effects may act on individuals indirectly, leading to death, and more broadly act to shift the ecological balance of affected areas, resulting generally in the slow disappearance of desirable species (e.g. community domination of those of benefit to man) with possibly an increase in abundance of undesirable species. Such effects have been seen in the results of the well-studied 1969 West Falmouth spill of No. 2 fuel oil (Sanders et al. 1972, 1980). In an oil-polluted environment, some of the petroleum compounds will be in solution or in emulsion in the water column, while the heavier fraction may enter the benthic mud and may affect the benthic ecosystem for many years (Blumer and Sass, 1972; Sanders et al. 1972, 1980; Sanders, 1978).

It has often been suggested that man's input of oil to the marine environment can cause interference with the chemical signals that are vital to the lives of the animals that live there. These signals - the subject of present research - are used for feeding (both for prey detection and scavenging), mating, habitat selection, migration, and for emergency and escape situations. There also is mounting evidence both in plants (Müller et al. 1971) and in animals (Miller, 1976) that the mechanisms by which sperm cells are attracted to the egg is based on chemical signals from the egg. Interference with these chemical communication systems undoubtedly has important consequences which may not be immediately obvious. Animals may not show clearly visible signs of locomotor difficulties, yet impairment of chemical communication and chemoreception may result in problems in feeding, finding mating partners, and in escaping from predators. Such subtle but potentially far-ranging effects of oil pollution have been explored here through behavioral analyses combined with neurophysiological analyses of chemoreceptors.

Since oil may alter any number of behavior patterns, it is important to select behaviors which are highly relevant to survival, are affected by oil, and which are amenable to laboratory testing. We have generally tried to adhere to the following sequence of experiments: 1) Nature studies or close equivalents to determine the general context in which the animal evolved and presently lives and to determine the sensitivity of the animal and of specific types of behavior to particular stresses in the environment. 2) Laboratory studies to quantify promising behavioral measures. 3) Detailed,

rigorously controlled laboratory tests to collect a data base that can serve to generate a model. 4) Verification of laboratory test results with field studies on the same behavioral effects. This paper will deal with the second and especially the third step of experiments on lobsters. These animals were chosen because of background data available and relevance to the ecosystem (i.e., point 1 in the sequence of experiments) and their ready availability and laboratory adaptability. Field verification has not been done.

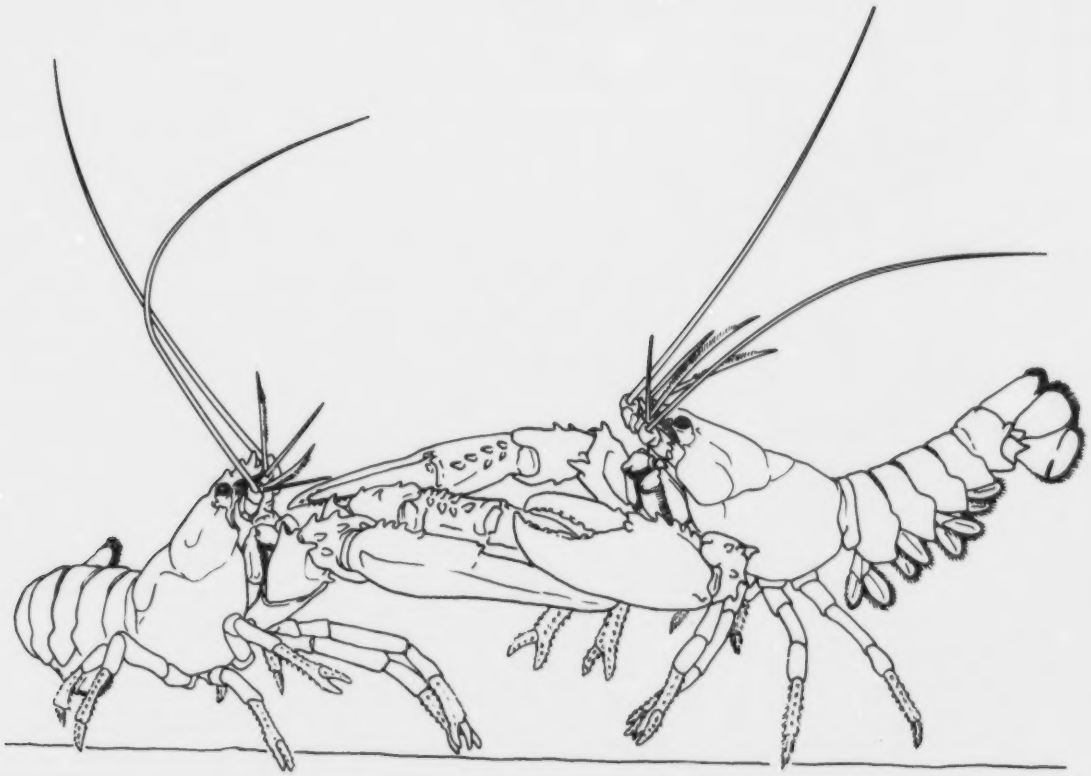
Interference With Chemoreception

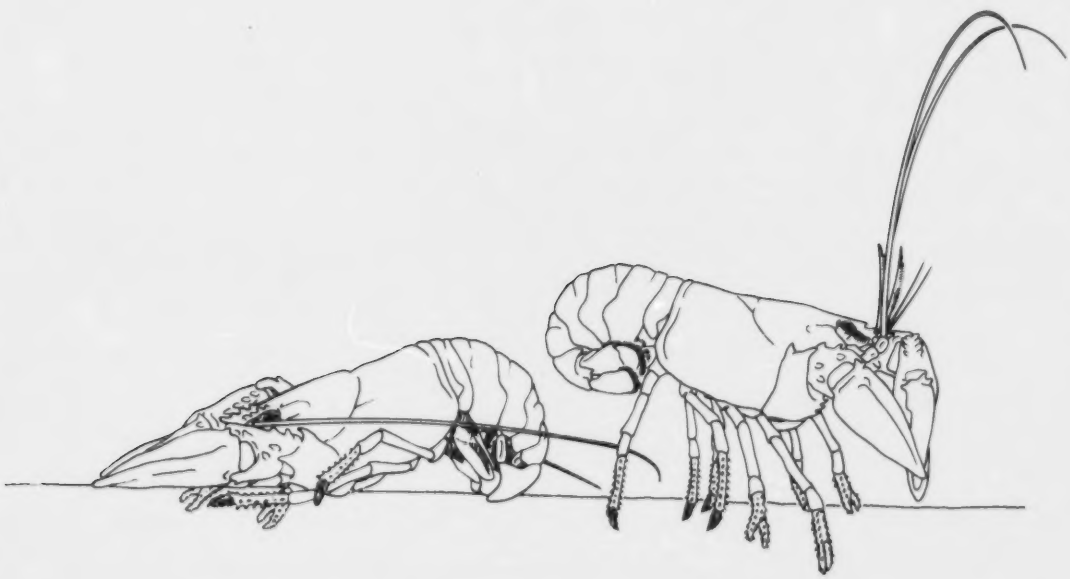
Speculation on the manner in which petroleum hydrocarbons interfere with chemoreception has appeared with a certain regularity in the literature, starting with Blumer (1970). Petroleum hydrocarbons are a mixture of organic chemical compounds, some of which are related to compounds such as pheromones and alarm substances, utilized by animals for their orientation and communication. These communication signals may have chemical features in common with compounds in petroleum, such as carbon skeleton, functional groups, volatility, and solubility (Jaenicke et al., 1974). One can thus envisage the scenario that these chemical look-alikes cam mimic or mask the reception of biologically important signals. Mimicked signals may result in "false alarm", i.e., animals may look for imaginary food or mates, or avoid predator danger where there is none. Alternatively, if their chemical signals are masked, animals cannot respond to them and may miss opportunities to feed, or mate, or escape. A third possibility less frequently mentioned is that animals may become subject to two competing signals (Atema and Stein, 1974), for instance, an attractant signal from food (or mate) and a repellent signal from the oil. In such cases chemoreception processes may occur normally but the animal may not be able to decide whether to feed or hide. Such delays may be more critical than apparent at first glance as even slight delays in responding to food can put an individual at a significant disadvantage when competing with an unimpaired conspecific or when seeking escape from predators.

Thus far, some cases of mimicked food attraction and delayed food responses have been observed in marine animals exposed to oil, as well as increases and decreases in alarm and attraction behavior (Mitchell et al. 1972; Takahashi and Kittredge, 1973; Walsh and Mitchell, 1973; Atema, 1976; Olla et al. 1980). Also, effects of oil on chemoreception itself have been documented (Atema et al. 1979a). However, whether changes in behavior are caused by oil-damaged chemoreception is not known. Studies examining oil interference with chemoreception are important to give us a better understanding of the potential effects of oil pollution. As the processes of chemoreception - although essentially not understood - are probably similar in all animals, interference with chemoreception or chemically mediated behavior also may prove to be one of the most sensitive and most general biological measures of low-level oil pollution. Much more obvious neuromuscular abnormalities appear at higher, although still sublethal, levels of oil exposure (Atema, 1977) (See Figure 1).

Choice of Species

The lobster, Homarus americanus, was chosen for the study of sublethal





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effects of low levels of petroleum in the environment for a number of reasons: a) it lives in and on the floor of inshore and continental shelf waters, areas now being leased for oil exploration and often affected by oil spills and chronic discharges. The benthic and shelter-dependent existence of lobsters insures that they will come in close contact with the bottom sediments, which appear to be most affected by petroleum contamination over the long term; b) in some areas it is the dominant benthic species; hence effects on the lobster population would be expected to have consequences at the level of the local ecosystem; c) a considerable amount of background biological data is available on this animal, plus some oil and drilling mud toxicity studies on various life stages, greatly facilitating the interpretation of results from highly controlled laboratory experiments; d) it supports an important commercial and recreational fisheries, with "the Maine lobster" almost standing as a symbol of the entire New England coast, and as a symbol of fine dining the world over; its decline would be a significant and highly visible problem with socio-economic consequences.

Context

Toxicity studies must be carried out under well controlled laboratory conditions to be able to document time/concentration effects of pollutant and to compare effects between species, between stages in their life cycles, and between seasons. To interpret with any confidence the results from behavioral laboratory toxicity studies, one must also understand the animal's natural environmental conditions. This work is underway for the American lobster (Karnofsky and Atema, 1979; Karnofsky, Elgin and Atema, in prep.). Field studies are nearly impossible to control. Therefore an intermediate step which can be employed successfully is suggested by our longitudinal studies which employ naturalistic large aquaria, where a small group of animals can be observed for extensive periods of time under semi-natural conditions (Atema et al. 1979b). Animals of various stages of development are tested with this technique. Long-term observations of behavior generate a bulk of data which must be analyzed for effects of size, sex, season, stage in the molt cycle, past experience, etc. (Atema et al., in prep.). Such basic information on the animal's biology is mandatory for interpretation of effects of pollutants on vital behavior such as mating and feeding. For example, what appears as an effect of oil exposure may actually be caused by a combination of pre-molt aggression and lack of food.

Choice of Behavior Bioassay

The components of our study on the effects of oil on lobster behavior are represented in a flow diagram (Fig. 2). Specifically, for this study we chose the odor orientation, bait localization, and feeding behavior of the lobster, Homarus americanus. In this study, using the water-accommodated fraction (WAF) of #2 fuel oil, we are concerned both with distance chemoreception of the antennules and dactyls, and with the contact chemoreception of dactyls and maxillipeds. Chemical senses on all three appendages control feeding behavior.

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Lobsters use chemical cues from their environment to direct a great number of vital behavioral responses such as feeding, courtship and larval settlement. We have studied the effects of pollutants on the two major chemoreceptor systems. The antennular system (i.e. smell) is normally used to alert the lobster to the presence of food and to identify other lobsters. The latter includes pheromone detection and is essential in social behavior. Antennules also convey important directional information and thus aid in spatial orientation of the lobster. Aesthetasc hairs on the antennules function to detect distant chemical signals in low concentrations. They are necessary for efficient odor localization (Devine and Atema, 1978, 1981), an activity facilitated by antennule flicking (Schmidt and Ache, 1979). Secondly, the dactyl and maxilliped chemoreceptor system (i.e. taste) is directly concerned with food evaluation and feeding behavior (Derby and Atema, 1981) and plays an additional role in odor orientation behavior (Devine and Atema, 1981). Chemoreceptor hairs on the walking legs are essential in recognizing and picking up food and bringing it into the mouth while hairs on maxillipeds test food palatability for ingestion. Walking leg receptors are equally sensitive as antennule receptors, and include specialist glutamate detectors (Derby and Atema, 1980) and alkaloid receptors (Reilly et al., 1979). Because chemoreceptor physiology is similar among marine crustacea and fishes, the results obtained with lobster chemoreception may be extrapolated with some confidence to other marine animals.

The first purpose of these experiments is to determine the range of #2 fuel oil exposures that affect the feeding behavior of lobsters without causing neuromuscular disturbance. The second goal of these experiments is to examine the effects on chemoreceptors in animals where sublethal behavioral abnormalities have been observed. With this approach one might be able to show whether the observed behavioral abnormalities are caused by oil-induced malfunction of the chemoreceptors or by other factors, such as general neurotoxicity and negative motivation. "Motivation" effects were surmised from earlier studies (Atema and Stein, 1974), where the main sublethal effects of crude oil exposure were seen in those phases of feeding behavior that are the least under chemosensory control.

In this report we present a detailed analysis of the effects of sublethal levels of No. 2 fuel oil (WAF) on the feeding behavior in nearly to just mature lobsters. We established the minimum exposure level for reliably observable effects and we determined to some degree how these effects may be related to measured effects on chemoreception. A preliminary report on procedures and pilot experiments has been published (Atema et al., 1979a).

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SECTION 2

CONCLUSIONS

The feeding behavior of lobsters, Homarus americanus, was repeatedly disrupted by 0.3 ppm No. 2 fuel oil, water accommodated fraction (WAF). The disruption began within 24 hrs, lasted the entire 5 days of exposure, and began to disappear in the 24 hrs following end of exposure. Only very slight effects of WAF exposure were seen at 0.1 ppm levels. Severe behavioral effects (spasms, cramped postures, unresponsiveness) occurred at 1.5 ppm levels beginning within 4 hrs. A 30-hr exposure at 1.5 ppm caused feeding disruption for over 5 days post-exposure.

Although chemoreceptors detect petroleum as a stimulus and although petroleum changes receptor responses to food odors, there is no reason to believe that interference with chemoreception is the (only) cause of observed behavioral disruption. Petroleum does appear to cause general neurotoxic effects which affect chemoreceptors as well as other neurons. At higher exposure levels , such as 1.5 ppm, this leads to the observed loss of motor coordination and sensory responsiveness.

In the context of an animal's natural behavior, ecology and life history, certain vital behaviors can be sufficiently quantified to serve as measurements of pollution stress. Interpretation of the ecological significance of findings of controlled bioassays requires knowledge of the animal's natural context. For instance, knowing the lobster's natural foraging methods and its use of chemoreceptors in feeding behavior provides important clues in the evaluation of laboratory-generated data, such as the potential effects of a change in motivational state on the lobster's ability to survive.

SECTION 3

RECOMMENDATIONS

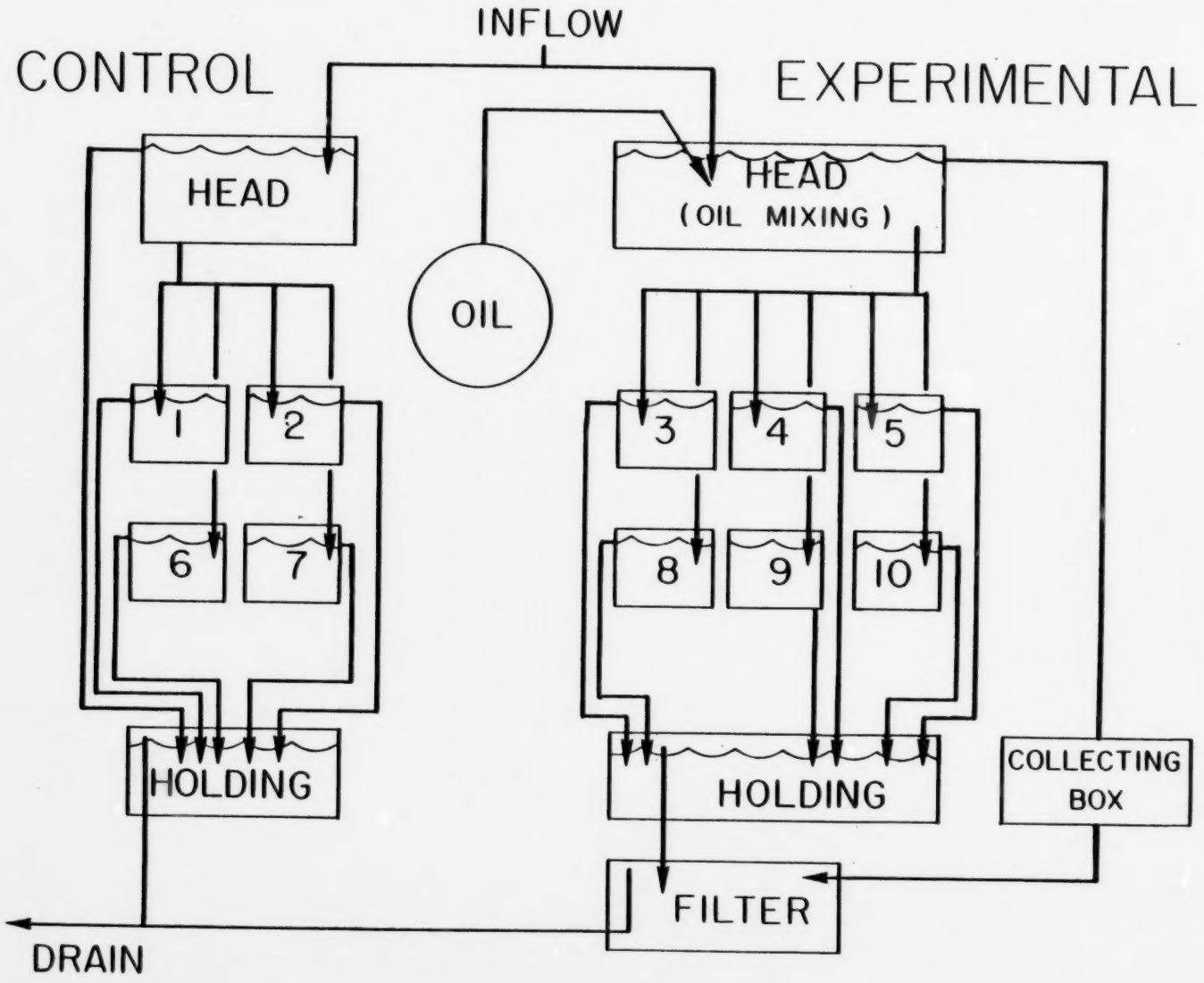
Based on the repeatedly obtained results that an oil concentration higher than 0.1 ppm No. 2 fuel oil (WAF) causes sublethal interference with feeding behavior, and adopting a safety factor of 10, it appears that water column levels below 0.01 ppm are safe for adult and subadult lobsters. This general statement must be accompanied by the following observations. Lobsters were not tested: a) during the stressful molting process, including the two weeks before and after molting; b) during their embryonic, larval and early juvenile stages; c) for other vital behavior such as substrate selection in juveniles or courtship and mating in adults; d) with exposures lasting longer than 5 days; e) under field conditions. Therefore, it is recommended that further investigations be undertaken before safety levels for chronic petroleum pollution are firmly established; in particular more information is needed on the effects of long-term exposures of larval stages. Specific behavior to study are hatching, molting, growth, feeding, phototaxis, settling, substrate selection, and shelter construction.

SECTION 4

MATERIALS AND METHODS

Lobsters, Homarus americanus, were obtained locally from the Woods Hole, Massachusetts, area. They were freshly trapped and claw-banded by ourselves or by commercial fishermen. Upon arrival in the laboratory their claws were unbanded and the animals were held individually in our holding facility in tanks (3.65 x 1.4 m) sectioned off (30 x 20 cm) with fiberglass-coated plywood dividers. The tanks were supplied with ambient flowing seawater and were maintained in ambient light. The animals were fed 2-5 times weekly with thawed frozen herring or freshly opened mussels (Mytilus edulis), depending on season. The lobsters used in this study ranged in carapace length (CL; measured from the rear of the eye socket to the posterior carapace margin) from 65-75 mm. This is approximately 1-2 molts under legal size (83 mm). Females in this size class are becoming sexually mature (Aiken and Waddy, 1980; Atema et al. 1979b); it is not known if males of this size are mature. Generally, animals which had molted 4-8 weeks prior were used in the experiments to avoid the effects of premolt behavior during observation. Equal numbers of females and males were used, evenly distributed between the controls and the experimentals. Naive, intact animals were used in each experiment, except experiment #8 where animals without antennules were used; the antennules were removed as part of experiment #7.

The behavioral experiments were conducted in a continuous flow-through dosing system, using the design of Hyland et al. (1977). The flow-through system (Fig. 3) consisted of 2 head tanks, one control and one experimental. The experimental head tank, 2.4 x 0.3 x 0.25 m, was fitted with three baffles to aid in the layering of the oil after the initial mixing. Its seawater inflow was 4 ± 0.2 l/min. Oil was introduced via a syringe pump (Sage Instruments, Model 341A) at a fixed rate into the center of a fast jet of seawater, causing rapid emulsification. The overflow of the head tank was skimmed off into a collecting tank where the oil layer was siphoned off occasionally. From the head tank the oil-water mixture entered individually six 100-liter lobster tanks (tanks 3, 4, 5, 8, 9, 10). The overflow from the individual tanks entered a holding tank where other lobsters were oil-exposed for the neurophysiological studies. The overflow from the collecting box and the holding tank entered an acrylic-fiber filter box where oil was removed before the water entered the drain. The control head tank (1.2 x 0.3 x 0.25 m) supplied four 100-liter lobster tanks (tanks 1, 2, 6, 7) and had an inflow of 2.6 ± 0.15 l/min. Physical constraints prevented the use of 6 control tanks. Inflow to individual tanks, both experimental and control, was kept between 400-460 ml/min. Individual tanks were made of fiberglass with one front glass window for observation. Each tank was supplied with a glazed clay shelter and pebble substrate. Pipes in the entire water system were made of PVC. During experiments, water quality (salinity, temperature, pH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FILTER
DRAIN

and O_2 content) was measured every other day and flow rates to individual tanks were measured every day. Ammonia was measured only during the first experiment. Levels were below detectable limits of the Solorazano method. Between experiments the entire system was cleaned with running hot water for several hours followed by flowing seawater for a few days. The pebble substrate was replaced for each experiment.

Experimental Design

The experimental design involved both internal and external controls to minimize interpretation problems due to individual variation in the behavior of these animals. As external controls, a group of four individuals was observed in parallel with the six experimental individuals and subjected to all experimental conditions except oil dosing. A pre-exposure (PRE) period of 5 days served as an internal control in which all animals including external controls were observed. During the immediately subsequent "OIL" period, which started at 10 AM of the 6th experimental day, the experimental group was exposed to a predetermined level of Exxon No. 2 fuel oil for 5 days. The oil was obtained from EPA, Environmental Research Laboratory, Narragansett, RI. To measure lobster recovery, a third 5-day "POST" period was added after oil exposure was halted at 10 AM on the 11th day. During the POST period, regular observations continued for all 10 animals. Thus, each experiment lasted 15 consecutive days. Oil concentrations were measured daily for control and experimental tanks, as described later. Each experiment was statistically analyzed separately for significant changes between "PRE", "OIL", and "POST" periods for five different aspects of feeding behavior.

Nine experiments will be described. Over all nine experiments, water temperatures varied from 8^{o}-23^{o} C during the April-November study period. Other experiments attempted at colder tamperatures were unsuccessful: lobster feeding behavior was too slow and unpredictable to give reliable results. See Table 1 for a summary of the experiments.

Behavior

In each experiment, two male and two female lobsters served as controls and three males and three females as experimentals. The animals were selected for close size match, put in the individual tanks in the flow-through system, and fed twice daily until all animals were feeding normally. This acclimation period varied from 1-2 weeks. During the whole experiment lobsters were observed twice daily in the early morning (7-9 am) and late afternoon (4-6 pm). One observer recorded behavioral units following food introduction and a second observer timed the phases of feeding behavior. One-minute behavioral observations were followed by the addition of food, which was lowered on a string from the right or left front corners, alternately. Then behavior was observed for an additional 10 min period or until the lobster grasped the food in its maxillipeds. Apart from general behavior (about 50 different postures and movements, Table 2), the times for ALERT (first observable response to food), WAIT (period between alert and leaving shelter to search), and SEARCH (time after first leaving shelter until food grasped with maxillipeds) were recorded. The moment the food was grasped by the maxillipeds, HIT, was recorded. Finally, it was noted if the animals

TABLE 1. SUMMARY OF OIL EXPOSURE CONCENTRATIONS AND BEHAVIORAL OBSERVATIONS OF NINE EXPERIMENTS

Exp #	Mean (±SEM) Oil Level (ppm)* Control	Exper.	Mean Temp (^{O}C)	Season (Mo/Yr)	Effects on Behavior	Comments
(1)	.027(.003)	.095(.010)	23	Aug 77	None	1 + 5 days of oil
(2)	.021(.008)	.117(.004)	21	Jul 78	Longer "Wait" AM + PM	
(3)	.021(.003)	.124(.010)	17	Jun 77	None	Oil low on day 9
(4)	.077(.008)	.238(.017)	19	Sep 78	Slower "Alert" + "Total" AM + PM; 2-4 days "No Hit"	Oil high PRE Control Oil low on day 8
(5)	.057(.005)	.284(.007)	20	Sep 77	Slower "Hit" + "Total" PM; 1-4 days "No Hit"	
(6)	.058(.003)	.506(.072)	9	Apr 78	Slower "Alert" + "Hit" PM; 1-3 days "No Hit", 1-6 days "No Eat"	Oil level not known for days 7-10
(7)	.056(.006)	.435(.032)	16	Oct 77	Slower "Alert" + "Hit" + "Total" PM; 2-4 days "No Hit"	Antennules being cut Oil peak on day 7
(8)	.05 (.001)	.250(.006)	12	Nov 77	Slower "Alert" + "Wait" + "Total" PM; 2-5 days "No Hit"	No antennules
(9)	.05 **	1.5 **	10	Apr 77	Gross neuromuscular effects within 30 hrs; more than 5 days recovery required post-exposure	Oil stopped in 30 hrs

* Daily fluctuations are described in Fig. 6.

** Because of short exposure period in experiment #9, standard error was not determined.

TABLE 2. BEHAVIORAL DESCRIPTIONS USED IN FEEDING EXPERIMENTS *

	Before Food Introduction	After Food Introduction
Stance	Alert	dig
	Rest Position	fake feed
	Defensive Posture	hesitate
	Hangout	jab
	High	manipulate pebbles
	Low	pounce
	Seizer 1 tight closed	rear
	2	return
	3	run
	4 wide open	snap
	Crusher 1 tight closed	tail flip
	2	touch
	3	turn
	4 wide open	walk
	Claws in - defensive	
	Claws out - relaxed	
Rate	Antennules - slow	
	medium	
	fast	
	burst	
	Fan - slow	
	medium	
	fast	
	burst	
Grooming	Antennae - forward	
	back	
	1 forward, 1 back	
	wipe	
	wave	
	point	
	Antennules - wipe	
	point	
	Maxillipeds - loose	
	open	
	wipe	
	Dactyl - wipe	
	sway	
	feel	
	Body - groom abdomen	
	groom claws	
Burrow cleaning	- bulldoze	
	rake	
	pleopod fan	
Barricade	- state of shelter	

* See also Figure 4

actually ate the food after grasping (EAT). A sample of a 5-day data sheet is shown in Fig. 4.

Oil Chemistry

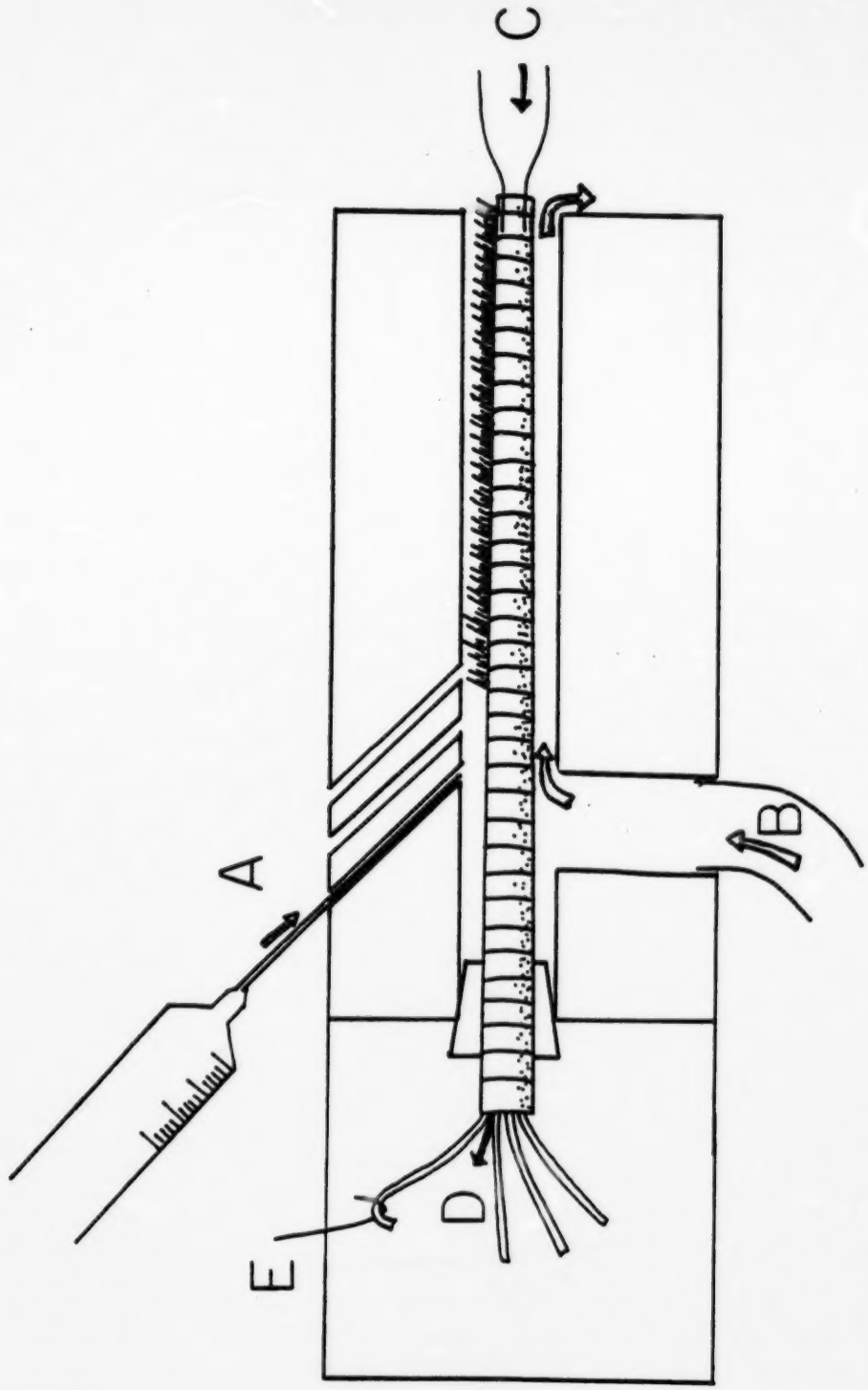
Infrared (IR) and ultraviolet (UV) spectroscopic and gas chromatographic (GC) analyses were performed on water samples taken daily from the lobster tanks to determine 1) the concentration of hydrocarbons in the water column, and 2) the general type of hydrocarbon present. Daily concentrations were monitored primarily by IR. Since IR analysis was done in Narragansett, quick spot checks were made using UV to determine the initial dosing levels during the experiment. For IR analysis two liter (2 l) samples were extracted with 50 ml CCl_4. Absorbance was measured at 2926 cm^{-1} and concentration was determined using as standard the same fuel oil, Exxon No. 2. The following sampling schedule was used to determine both the concentration and full time course of exposure: one or two days pre-oil, 12 hr after start of oil injection, daily for the next 4 days of exposure, and daily for 2 days post-exposure. For UV analysis, hydrocarbons were extracted with CH_2Cl_2. Their concentration was estimated using absorbance at 295 um, an absorbance maximum found in Exxon No. 2 oil. For GC analysis, six liter (6 l) water samples were taken before and during oil exposure from one control and one experimental tank of each experiment. These samples were extracted with 300 ml CH_2Cl_2 and the extracts passed through columns of Na_2SO_4 (10 cm x 1 cm each). The solvent was exchanged with n-hexane and the samples submitted to GC analysis. This analysis was done at the EPA Laboratory at Narragansett.

Neurophysiology

To measure neurophysiological activity of chemoreceptors and the possible effects of oil exposure on receptor cell performance, the lateral (in Exp. #7, the medial also) flagellum of the antennule of a lobster was cut off and placed in fresh seawater. The cut proximal end was inserted through a Sylgard cork. Three to four cuticular rings were removed. The distal tip was cut and the antennule placed in a lobster saline bath in the stimulation-recording chamber (Fig. 5). A micropipette was inserted snugly into the distal tip, and perfusion with oxygenated lobster saline started within 10 minutes after the removal of the antennule from the animal. Test chemical stimuli were injected into the continuously flowing seawater bathing the antennule. Recordings were made by picking up a small nerve bundle with a platinum electrode. The signal was amplified via a Tektronix Type 122 preamplifier and displayed and recorded on conventional recording equipment for later analysis. Control antennules were taken from animals in our general holding facility (ambient seawater) or from experimental animals in the flow-through holding tank before oil exposure. Experimental antennules were taken from animals in the flow-through holding tank during and after oil exposure.

To document the direct effects of No. 2 fuel oil on lobster antennular chemoreception, we used a test protocol exposing the antennule preparation to the following stimuli: (1) mussel juice; (2) No. 2 fuel oil, 1 or 3 ppm; (3) mussel juice plus oil; (4) artificial seawater; and (5) mussel juice. Artificial seawater was made according to the MBL formula: 420 mM NaCl, 9 mM KCl, 9 mM $CaCl_2 \cdot 2\ H_2O$, 23 mM $MgCl_2 \cdot 6\ H_2O$, 26 mM $MgSO_4 \cdot 7\ H_2O$, 2 mM $NaHCO_3$ (pH 7.3).

Experiment _____ Lobster _____ Day Time	Day 1	Day 2	Day 3	Day 4	Day 5
Stance					
Rate: Antennules					
Rate: Fan					
Grooming: Antennae					
Grooming: Antennules					
Grooming: Maxillipeds					
Grooming: Dactyl					
Grooming: Body					
Burrow Cleaning					
Barricading					
Comments:					
After Feeding					
Alert-Wait-Hit Comments:					


C
A
B
D
E

This was used to eliminate day-to-day variations in natural seawater. Stock solutions of mussel juice were made at one time and refrigerated. Mussel juice was made by homogenizing 10 g wet weight of Mytilus edulis tissue in 100 ml artificial seawater. The suspension was centrifuged at 27,000 xG for 20 min and the pellet discarded. The supernate was frozen in small aliquots until needed. Oil mixtures (10 ppm WAF No. 2 fuel oil) were made at the start of each recording session due to the lability of the oil-water suspension. Dilutions were made from this stock solution. Stimulus concentrations were varied from 1-3 ppm, although occasionally 10 ppm was used in preliminary recordings.

This protocol allowed us to compare the response to stimulus 1 with the response to stimulus 5 for nerve fiber damage or fatigue, or for lasting effects of the prior oil test stimulus. Stimulus 2 and stimulus 3 were used to determine a) whether lobster antennules can detect oil as a chemical stimulus and b) if the presence of oil changes the response to mussel juice. Stimulus 4 was used to determine the sensitivity of the preparation to a chemically neutral stimulus; this measures mechanoreceptor and spontaneous activity which can then be substracted from the responses observed in other tests in order to detect the magnitude of purely chemosensory responses.

SECTION 5

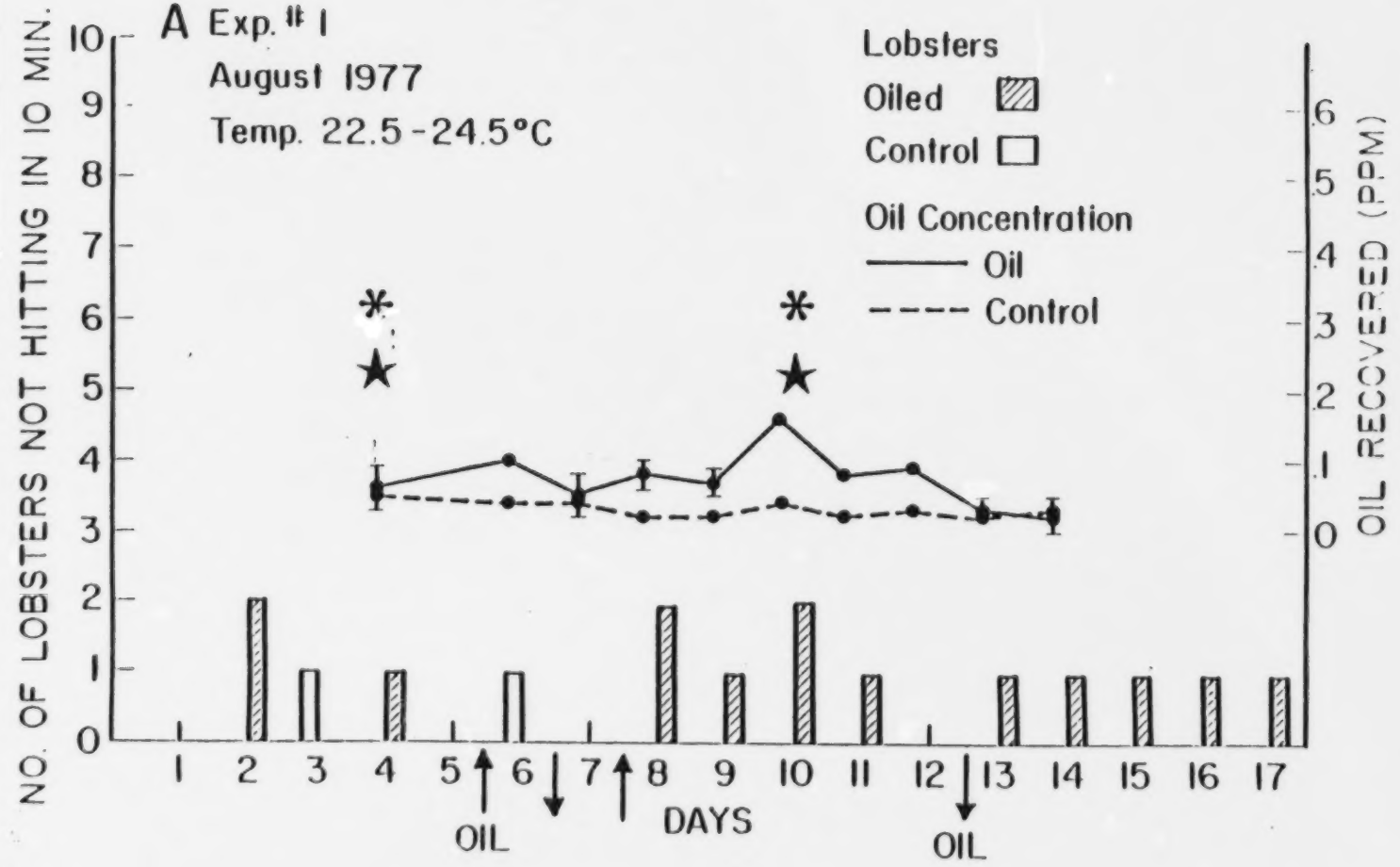
RESULTS

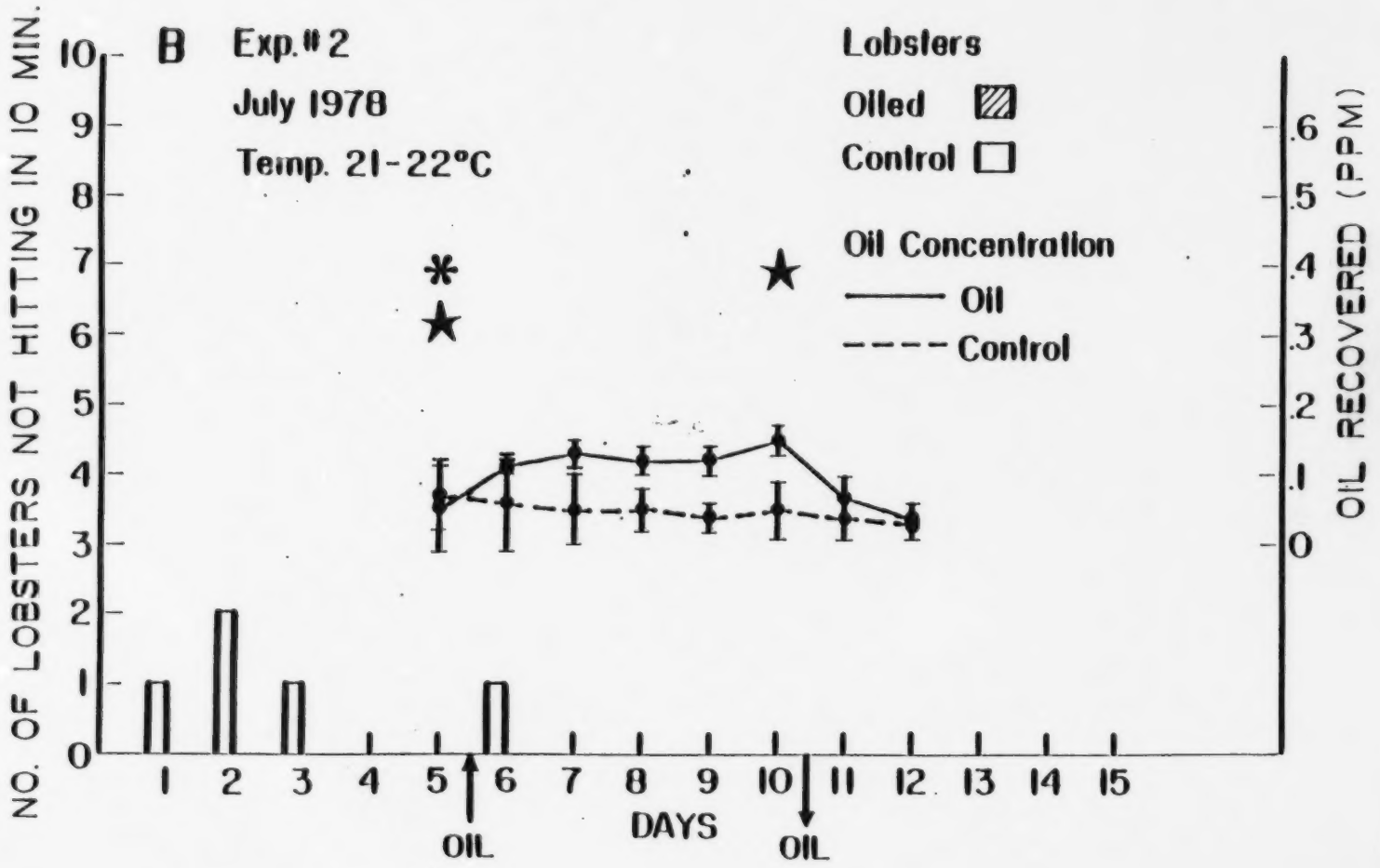
BEHAVIOR

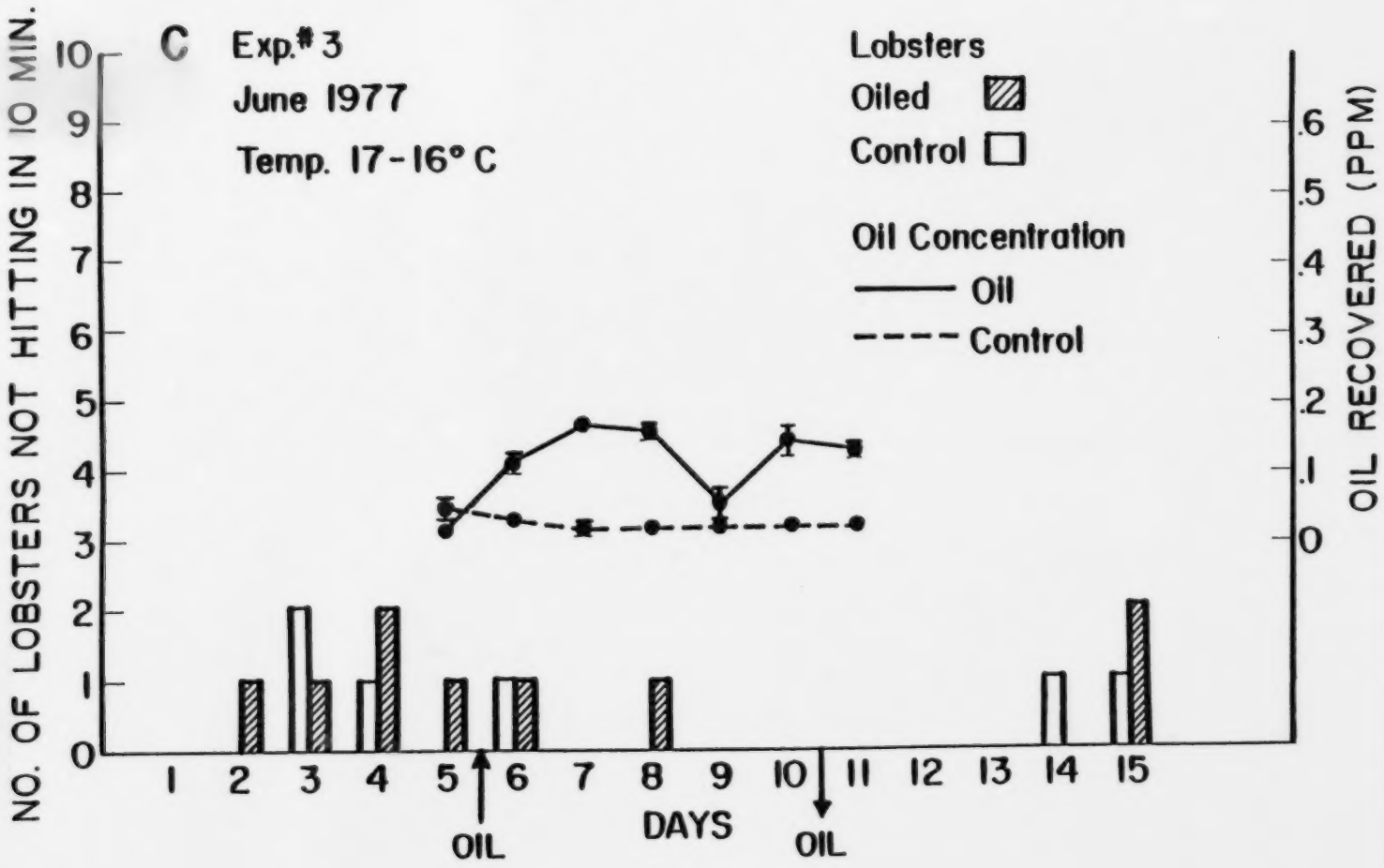
The nine experiments break down into three groups: a group of three (#1, 2, 3) with "low" exposure levels of about 0.1 ppm, a group of three (#4, 5, 6) with "high" exposure levels of about 0.3 ppm, with an additional two experiments in which antennules were being removed (#7), or had been removed (#8), and one experiment (#9) at higher exposure levels (1.5 ppm). Table 1 gives a summary of the important data and the results. Lobsters exposed for 5 days to 0.1 ppm (WAF) No. 2 fuel oil showed no significant changes in feeding behavior. Lobsters exposed 5 days to 0.3 ppm oil with or without antennules showed consistent delays in feeding behavior, often resulting in no feeding within 10 minutes. Higher (1.5 ppm) exposure resulted in gross neuromuscular defects within 30 hrs. Each experiment is graphically summarized in Fig. 6, A-J. These figures show the recovered oil levels (IR determination) for exposed and control aquaria, and the ultimate result of sublethal oil exposure on feeding behavior, namely the number of lobsters that in 10 min. did not reach their food, expressed as "no hits" summed over morning (AM) and evening (PM) feeding observations.

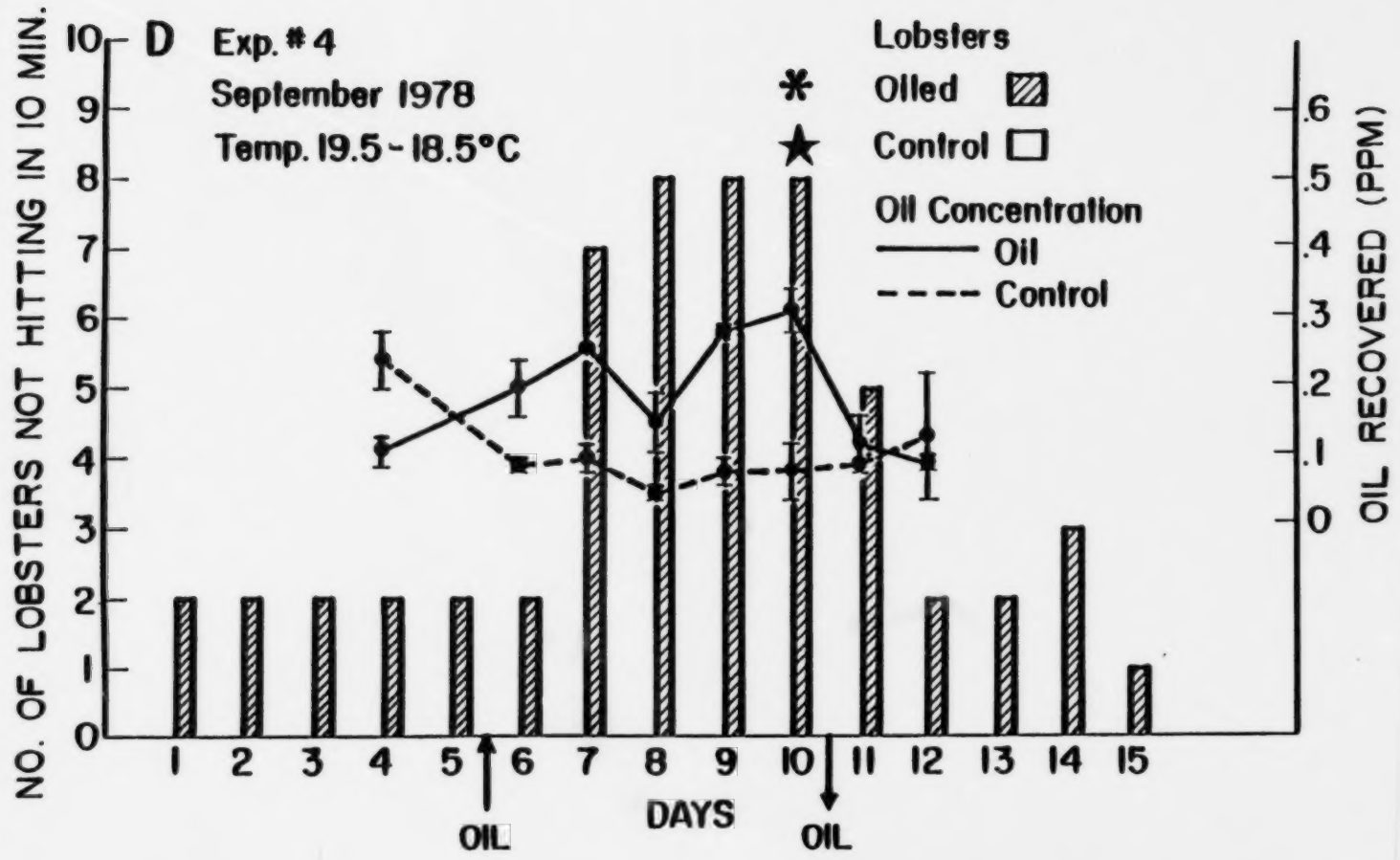
Number of lobsters not feeding in 10 min.

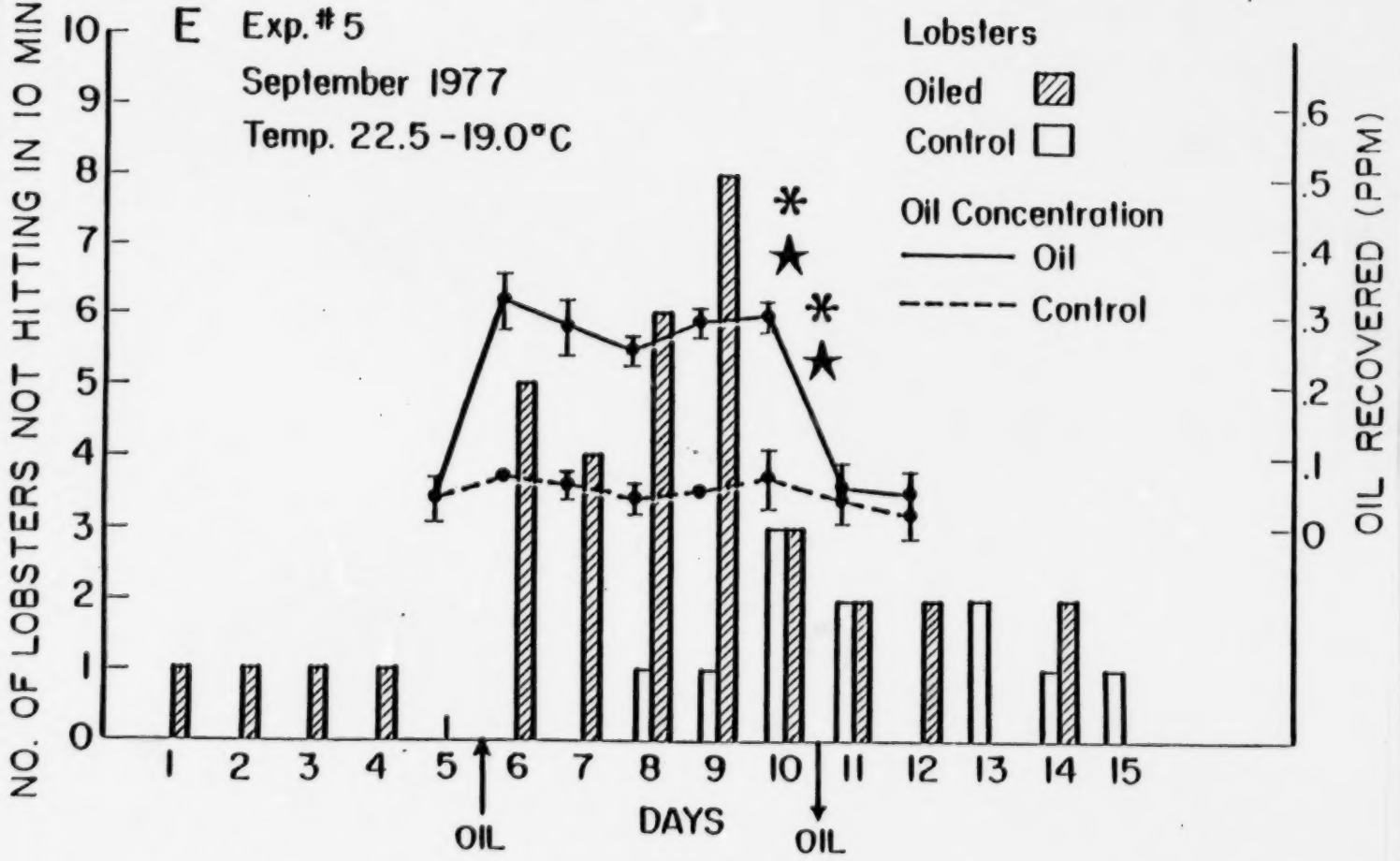
In most experiments (Fig.6, A-J) one finds one or two lobsters not interested in food either for one day (AM plus PM) or for several days in either the morning or in the evening. The latter apparently eat only once a day. Keeping this in mind, "no hit" scores of 1 or 2 are not abnormal and may occur in both control and experimental groups and in PRE, OIL and POST periods alike. Figs. 6, A-C show, that the low oil exposures (0.09-0.14 ppm) did not affect food finding within a 10-minute period. Figs. 6, D-F show that higher (0.24-0.3 ppm) oil exposures cause a great number of animals to not reach their food within 10 minutes. This may involve most animals of the experimental group, and sometimes both in AM and PM resulting in scores of greater than six. The "no hit" effect may last one day (day 11) after oil exposure, but not longer. Fluctuations in the oil concentration occurred within the 5-day exposure period in Experiments 1, 3, 4 (Figs. 6A, C, D); yet the oil dose effects appear to be very similar to those experiments having more consistent oil concentrations (Figs. 6B, E, H). The significance of oil concentration data is further discussed in the chemistry results section. The oil exposure in experiment #6 (Fig. 6F) after the first day of oil exposure could not be determined due to loss of samples, but is believed to have been around 0.3 ppm, since oil was added at the same rate of experiments #4, 5, 7, 8.

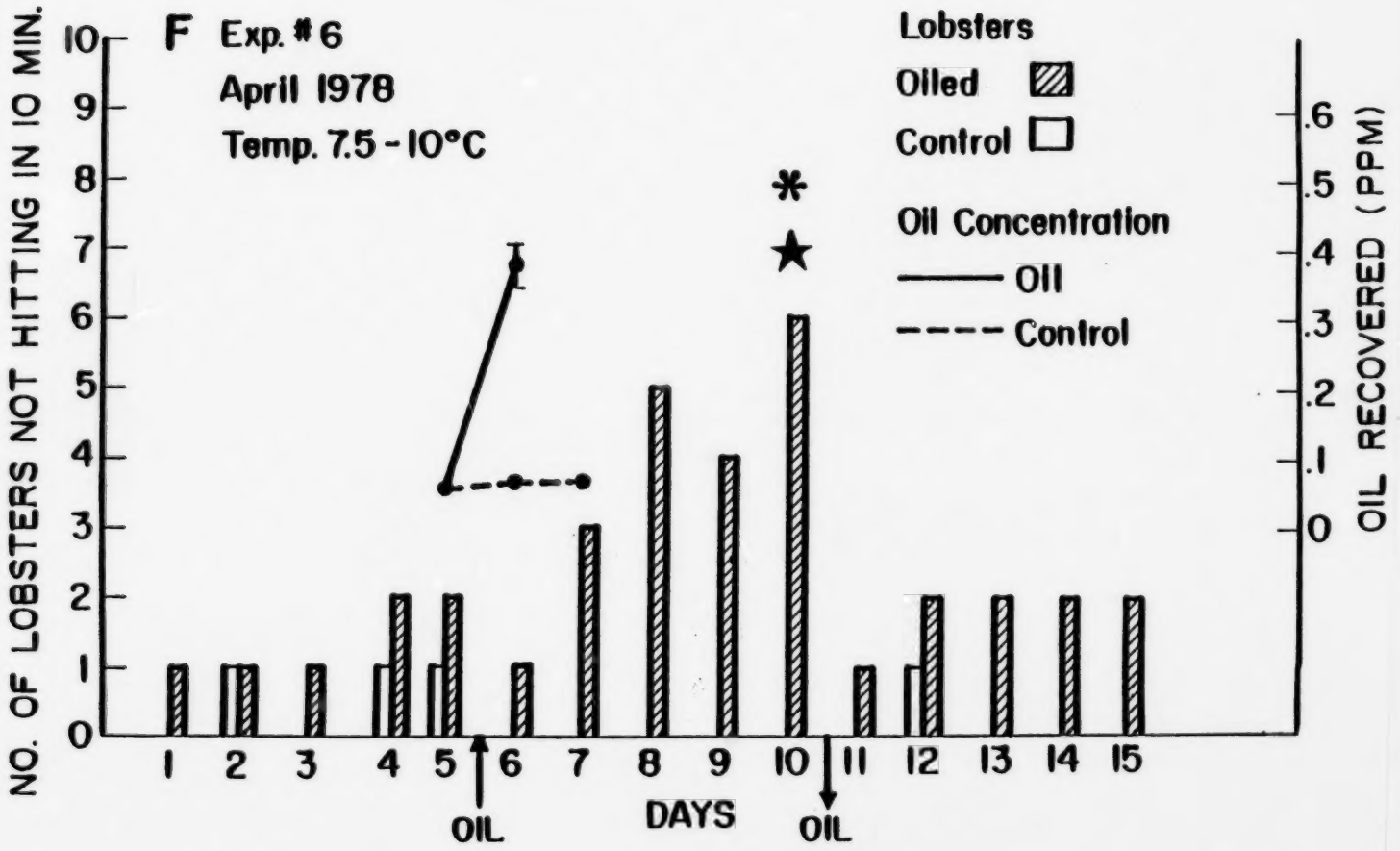

A Exp. # 1
August 1977
Temp. 22.5 - 24.5°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT HITTING IN 10 MIN.
OIL RECOVERED (PPM)
DAYS
OIL
OIL

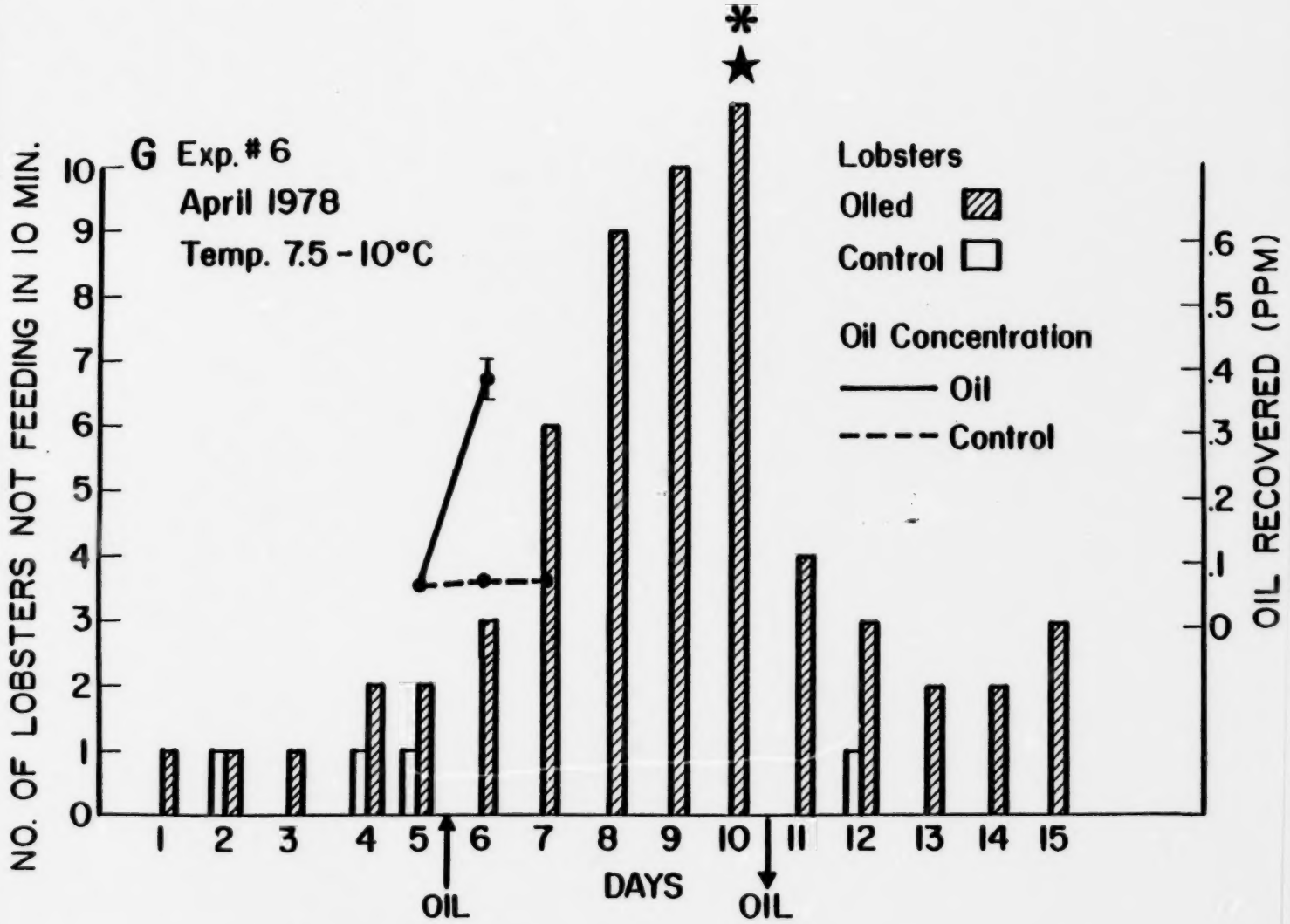

B
Exp.# 2
July 1978
Temp. 21-22°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT HITTING IN 10 MIN.
OIL RECOVERED (PPM)
DAYS
OIL
OIL
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
.1
.2
.3
.4
.5
.6

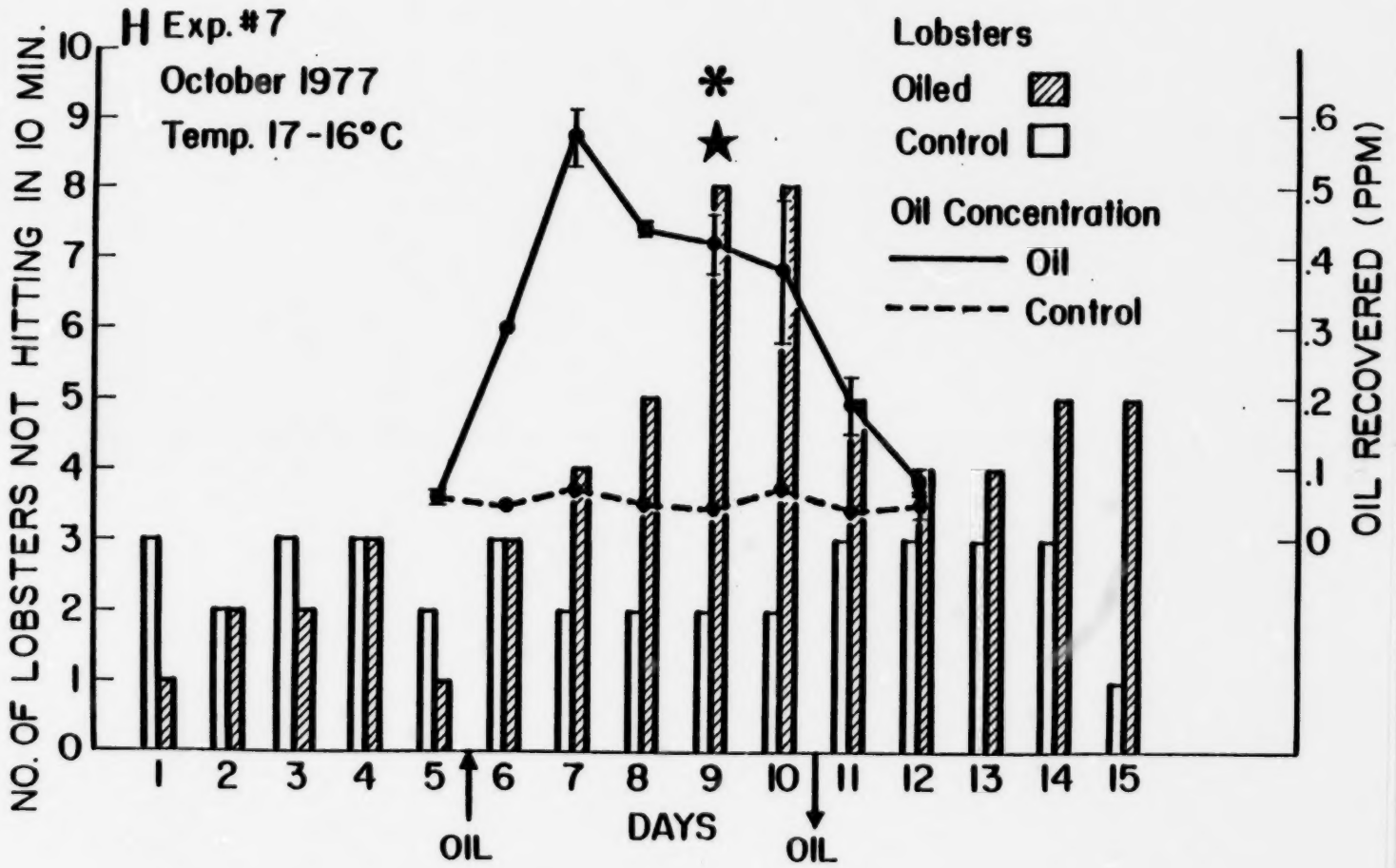

C
Exp.# 3
June 1977
Temp. 17-16° C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT HITTING IN 10 MIN.
OIL RECOVERED (PPM)
0
1
2
3
4
5
6
7
8
9
10
.1
.2
.3
.4
.5
.6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS
OIL
OIL

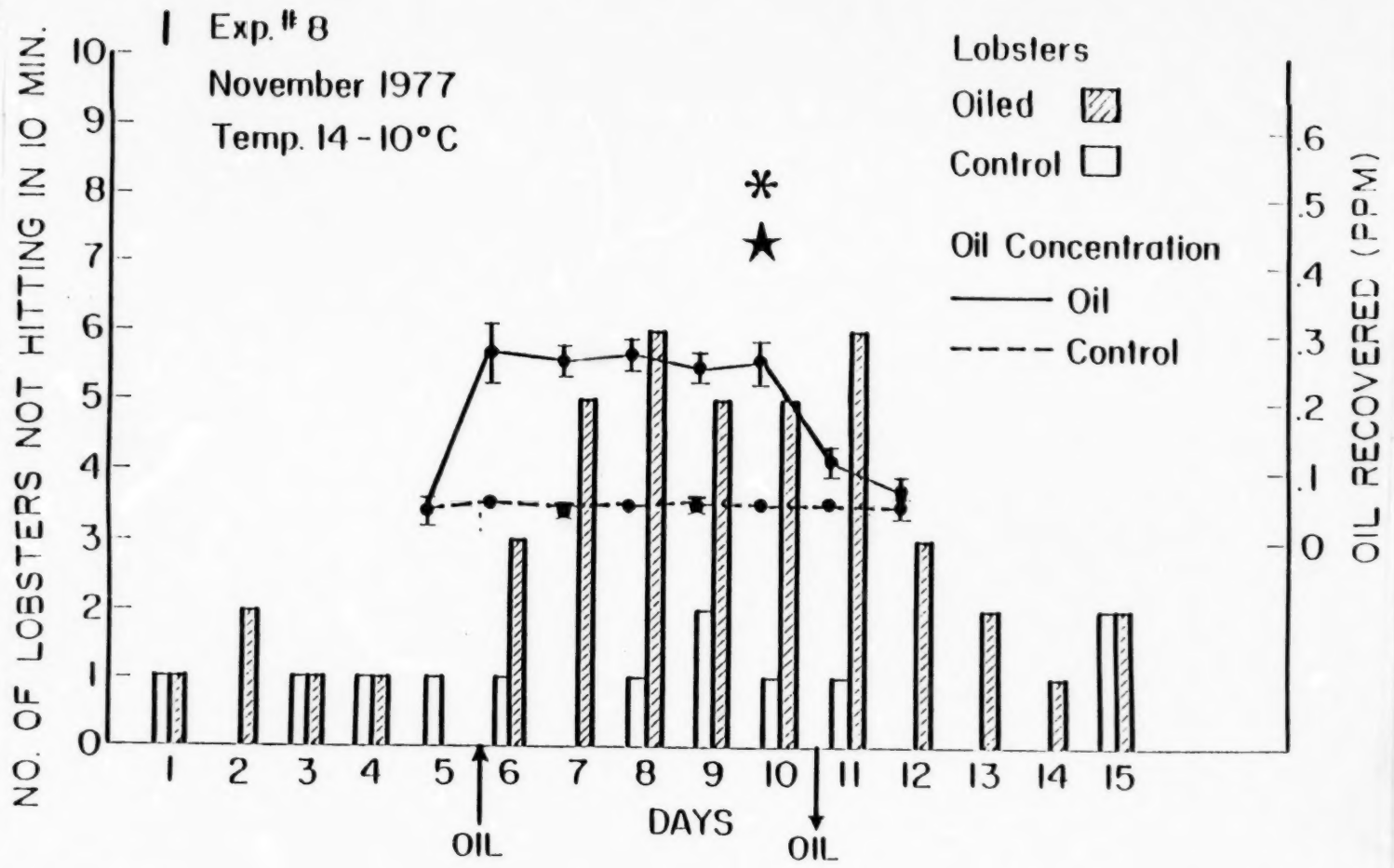

D
Exp. # 4
September 1978
Temp. 19.5 - 18.5°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT HITTING IN 10 MIN.
OIL RECOVERED (PPM)
DAYS
OIL
OIL

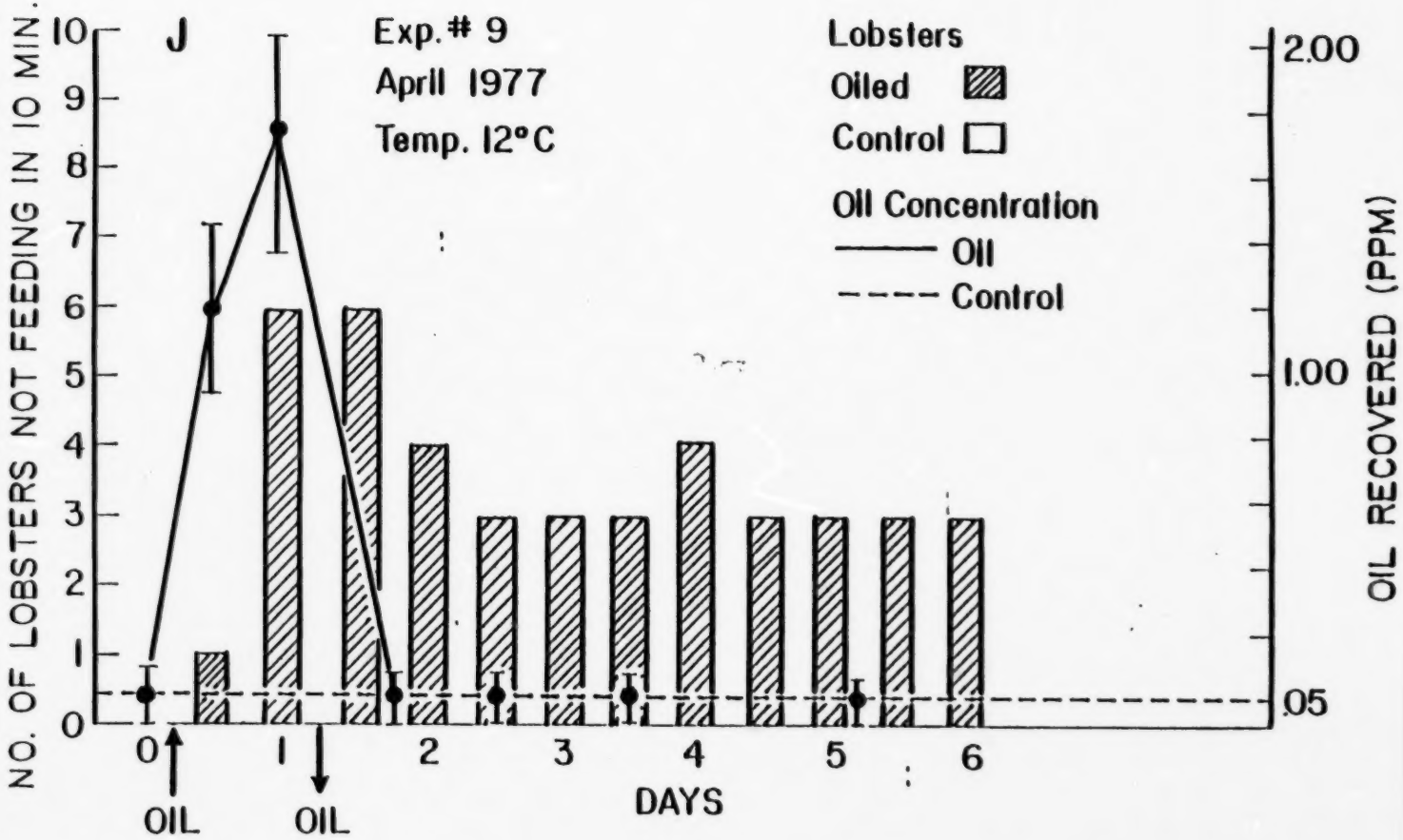

E
Exp. # 5
September 1977
Temp. 22.5 - 19.0°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT HITTING IN 10 MIN.
OIL RECOVERED (PPM)
DAYS
OIL
OIL
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
.1
.2
.3
.4
.5
.6


F Exp. # 6
April 1978
Temp. 7.5 - 10°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT HITTING IN 10 MIN.
OIL RECOVERED (PPM)
DAYS
OIL
OIL
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
.1
.2
.3
.4
.5
.6


G Exp. # 6
April 1978
Temp. 7.5 - 10°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT FEEDING IN 10 MIN.
OIL RECOVERED (PPM)
DAYS
OIL
OIL
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
.1
.2
.3
.4
.5
.6


H Exp.#7
October 1977
Temp. 17-16°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT HITTING IN 10 MIN.
OIL RECOVERED (PPM)
DAYS
OIL
OIL
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15


Exp. # 8
November 1977
Temp. 14 - 10°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT HITTING IN 10 MIN.
OIL RECOVERED (P.P.M)
DAYS
OIL
OIL
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
.1
.2
.3
.4
.5
.6


J
Exp.# 9
April 1977
Temp. 12°C
Lobsters
Oiled
Control
Oil Concentration
Oil
Control
NO. OF LOBSTERS NOT FEEDING IN 10 MIN.
OIL RECOVERED (PPM)
DAYS
OIL
OIL
0
1
2
3
4
5
6
10
9
8
7
6
5
4
3
2
1
0
2.00
1.00
.05

The unusually large number of "no hits" in experiment #7 (Fig. 6H), seen in control and experimental groups and during the PRE, OIL and POST periods, is believed to be the result of antennule removal (Devine and Atema, 1981). The effect of oil exposure appears superimposed on the lesion effects. One month later the same animals, which had then been without antennules for a month, were used in experiment #8. Now they responded not differently from intact animals to oil exposure (Fig. 6I), using the overall criterion whether or not the animal reaches its food within 10 minutes. Thus, the presence of antennules does not appear to mediate the changes in feeding behavior caused by oil exposure.

The only experiment done at a low temperature (#6) showed an additional effect. Although the number of "no hits" (Fig. 6F) was similar to that found in other experiments at a comparable oil exposure (Figs. 6D, E, H), most animals did not eat their food once they had grasped it (NO EAT, Fig. 6G), but animals were often seen an hour later still carrying uneaten food.

One additional experiment (#9, Fig. 6J) was carried out at a higher exposure level (1.5 ppm). After 30 hrs of oil exposure, the lobsters showed gross neuromuscular defects and oil inflow was stopped. In this experiment, post-exposure behavioral recovery was compared with pre-exposure behavior during the five-day recovery period. Experimental lobsters were slower in all phases of feeding than during the five-day pre-oil period ($p < 0.001$). Even five days after this 30 hr exposure to 1.5 ppm #2 fuel oil, half the lobsters did not feed within the 10-min limit (Fig. 6J). Behavior of control lobsters showed no significant differences between test periods. The animals during experiment #9 showed three levels of severity of oil effects, some animals being affected much more than others. A description of the three levels follows, as quoted from Atema et al. (1979a):

> "Most extreme effects" (two lobsters) - After 30 hours of oil exposure, these lobsters were found outside their burrows lying on their backs, pleopods twitching or still, tail half curled, walking legs twitching, antennae and antennules limp, gill bailers moving slightly. Occasionally the back and tail were arched and then curled. At times, attempts were made to right itself. Body jerked after food entered into tank. No recovery in five days. (See Fig. 1)
>
> "Moderate effect" (three lobsters) - These animals were generally out of their burrows in two alternating stances. In one, the tail and head were down, the antennae were folded back, the antennules beat slowly, and the animal lay down low on the walking legs (Fig. 1). This appeared to be a resting position. Then the tail and head arched up, the claws were opened and held close to the body, the antennae and antennules were held straight up and together, animal stood high on walking legs, and made frequent tail flips. Poor coordination was exhibited. Frequent aggressive lunges were made with open, raised seizer claw, or jabs with both claws at no obvious target (Fig. 1). Lobsters were unaware of the presence of food, at times walking right over it without responding. If they did pick up the food, they continued to wander aimlessly around the tank with the food clutched tightly in the maxillipeds.

Recovery in five days was almost complete in two animals; one animal did not recover.

"Light effects" (one lobster) - This animal remained in its burrow but was high on its walking legs, "spider" position and shaking (Fig. 1). There were sporadic alerts not necessarily related to food. It exhibited no search. After two days recovery, there were slow hesitant approaches to the food with tail flips at the slightest irregularity. At the end of five days there was complete recovery.

Oil Effects on Presence of Feeding Behavior Phases

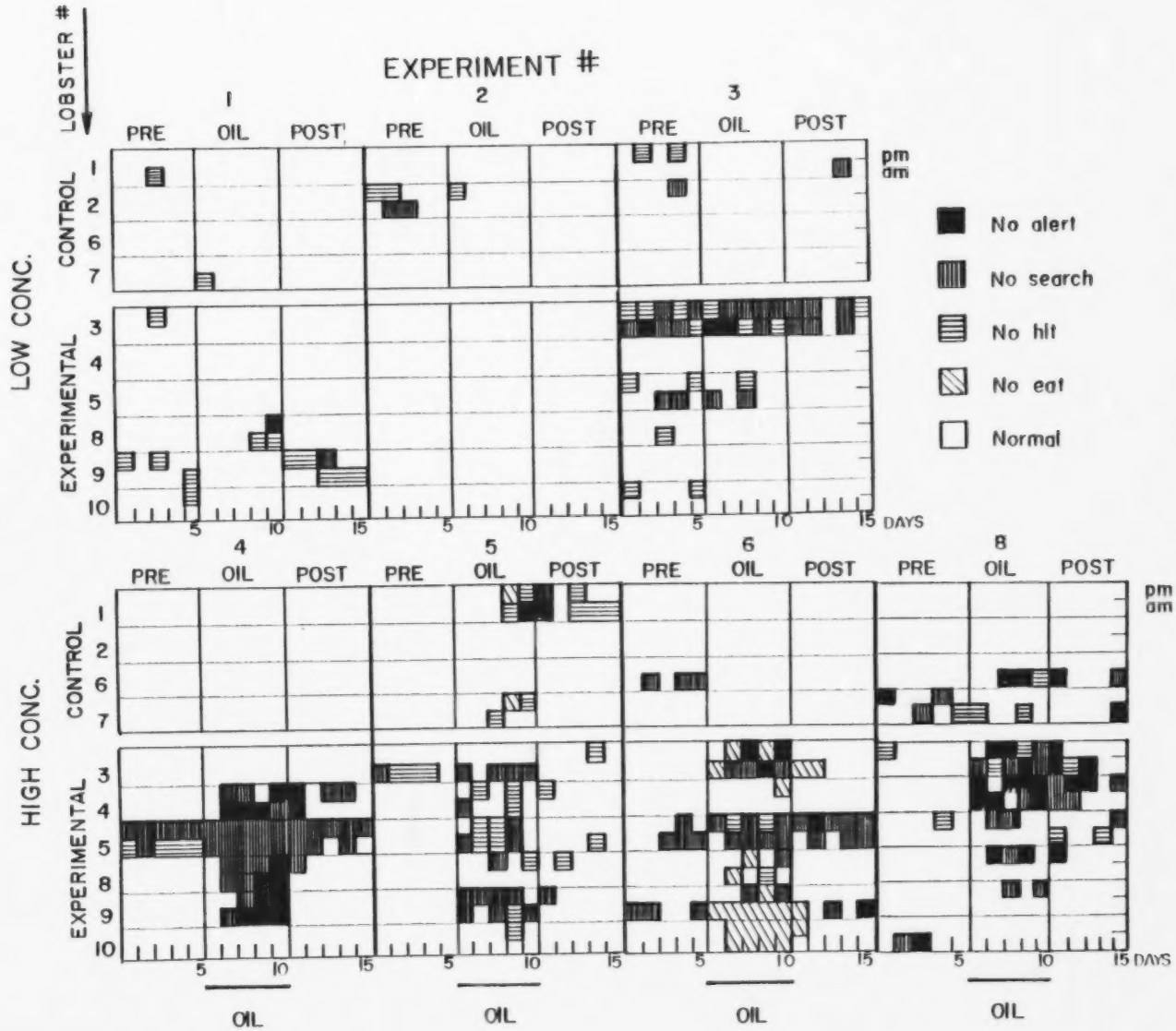
The parameter "no. of lobsters not feeding in 10 min" gives a general description of the ultimate oil exposure effect observed in this study. To approach a possible mechanism of oil interference, a more detailed analysis was made. It shows the oil effects on the different phases into which the behavioral sequence of feeding was divided, illustrating in a graphic representation at what phase the feeding sequence was broken off for each animal at each observation (Fig. 7). The normal sequence is ALERT, WAIT, SEARCH, HIT, EAT. The least dramatic oil effect is NO EAT, followed by the more serious effect of NO HIT, then NO SEARCH, and finally NO ALERT. (NO SEARCH means that the animals only showed ALERT, etc.) Normal animals often do not wait during feeding. Hence, the WAIT phase was not used in the present analysis.

Fig. 7 shows that the low 0.1-ppm exposure (experiments 1-3) did not affect the feeding behavior sequence. (Note in experiment 3, there was reduction or breakdown of feeding behavior in several animals, especially in animal #3 during the PRE control period. This animal fed only 3 times out of 30 food presentations, i.e. in the PM and AM of day 13 and in the AM of day 15, both in the POST period.)

In contrast to the no effect observations with 0.1 ppm oil, the 0.3 ppm exposure (= HIGH CONC) caused clear behavioral deficits. Most (14 out of 18) experimental lobsters in experiments 4-6 showed breakdown of feeding behavior during exposure, with the effects usually extending into the POST period.

In experiment 4, all control animals fed normally, and 3 out of 5 experimental animals showed almost complete absence of feeding behavior, including frequent lack of alerting. These effects were generally seen after one day of exposure and, in animal #6, lasted for four days in the POST period. Responses of lobster #5 in experiment 4 were not counted because of limited feeding activity during the PRE period. Two other lobsters (#3 and #10) showed no oil effects.

In experiment 5, control lobsters fed normally, except lobster #1 which had feeding problems on days 9, 10, 11, 13 and lesser on days 14 and 15 despite the fact that no oil was present. The experimental animals showed NO SEARCH and NO HIT immediately after oil exposure started. This generally ended at, or one day after, oil exposure stopped. In this experiment lobster #3 was not counted since it failed to "Hit" its food on four PM observations in the PRE control period.


EXPERIMENT #
LOBSTER #
LOW CONC.
HIGH CONC.
CONTROL
EXPERIMENTAL
PRE
OIL
POST
pm
am
DAYS
No alert
No search
No hit
No eat
Normal

In experiment 6 all control animals fed well and all experimental animals were more or less seriously affected by oil. Lobster #3 showed the clearest effects; lobster #5 did not recover in the 5-day POST period; and lobster #4 showed hardly any effect of oil.

The experiment (#8) with animals without antennules showed effects of oil exposure similar to those of the other 0.3 ppm exposed animals. Results of experiment 7 are not presented because the effects of cutting antennules confounded effects of oil exposure. Also, experiment 9 was not subjected to further analysis.

Fig. 7 gives a clear visual impression of the effects of 0.3 ppm oil exposure on the presence or absence of phases in the feeding behavior sequence. The G-test ($\alpha = 0.05$) was used to quantitatively compare the presence of phases between PRE and OIL periods for CONTROL and EXPERIMENTAL groups. The 3 low exposure experiments (1, 2, 3) were lumped together, as were the 3 higher exposure experiments (4, 5, 6). CONTROL and LOW EXP groups showed no significant changes between PRE and OIL periods. Only in the HIGH EXP group was a change (decrease in feeding activity during OIL) shown in the number of completed phases of feeding behavior, confirming what Fig. 7 suggests.

Oil Effects on Duration of Feeding Behavior Phases

One step further in the analysis was to test for oil effects not just on presence but on the duration of the feeding behavior phases. The ALERT, WAIT, SEARCH and TOTAL times (in seconds) were compared between PRE and OIL and between PRE and POST periods in the 7 experiments (excluding experiments 7 and 9), using the Bonferroni test ($\alpha = 0.05$). In this analysis lobsters which did not feed during the PRE control period were not counted. A comparison of PRE-OIL data (Table 3) shows slower behavior was observed consistently and only during the HIGH CONC Oil periods, including experiment 8. Differences between the four experiments (including #8) are small. In experiments 4 and 5 both AM and PM feeding were equally slower, but in experiment 5 ALERT was not significantly different; in experiments 6 and 8 only PM feeding was slower. The CONTROL in experiment 2 showed significantly faster feeding behavior, particularly shorter WAIT, in both OIL and POST periods.

Comparing PRE and POST periods, few significant differences are seen, indicating that behavioral recovery is complete in five days even for the high exposures (0.3 ppm). It should be remembered that the "POST" period represents the recovery mean of all animals over a 5-day period; individual animals generally required one day to recover and some even four days (Fig. 7).

Analysis for Sensory and Motivational Factors

The previous two analyses demonstrate that 0.3 ppm oil exposures affect the length and presence of phases of feeding behavior, often resulting in not feeding and sometimes in absence of any observable alert behavior. Fig. 7 also shows that some animals are seriously affected while others are less or not at all. Furthermore, feeding in nearly all animals is affected differently on different days, ranging from normal behavior to no search at all. This suggests that oil affected the motivation to search and eat, rather than causing a systematic neurosensory deficit, which would have affected all

TABLE 3. SIGNIFICANT CHANGES IN FEEDING BEHAVIOR *

		LOW						HIGH				NO ANT.	
		1. (Aug 77) 23° C		2. (Jul 78) 21° C		3. (Jun 77) 17° C		4. (Sep 78) 19° C		5. (Sep 77) 20° C		6. (Apr 78) 9° C		8. (Nov 77) 12° C	
		Cont	Oil	Cont	Oil	Cont	Oil	Cont	Oil	Cont	Oil	Cont	Oil	Cont	Oil
PRE:OIL															
Alert	pm	–	–	–	–	–	–	–	S	–	–	–	S	–	S
	am	–	–	–	–	–	–	–	S	–	–	–	–	–	–
Wait	pm	–	–	F	–	–	–	–	S	–	S	–	S	–	S
	am	–	–	F	–	–	–	–	S	–	S	–	–	–	–
Hit	pm	–	–	–	–	–	–	–	S	–	S	–	S	–	S
	am	–	–	F		–	–	–	S	–	S	–	–	–	–
Total	pm	–	–	–	–	–	–	–	S	–	S	–	S	–	S
	am	–	–	F	–	–	–	–	S	–	S	–	–	–	–
PRE:POST															
Alert	pm	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	am	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Wait	pm	–	–	F	–	–	–	–	–	–	–	–	–	–	–
	am	–	–	F	–	–	–	–	–	–	–	–	–	–	S
Hit	pm	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	am	–	–	F		–	–	–	–	–	–	–	–	–	–
Total	pm	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	am	–	–	–	–	–	–	–	–	–	–	–	–	–	–

* The Bonferroni test at the $\alpha = 0.5$ level of confidence compared the changes in duration of the major phases of feeding behavior: Alert, Wait and Hit. "Total" refers to the sum of Alert, Wait and Hit times for overall effects. S = Slower, F = faster than in PRE period.

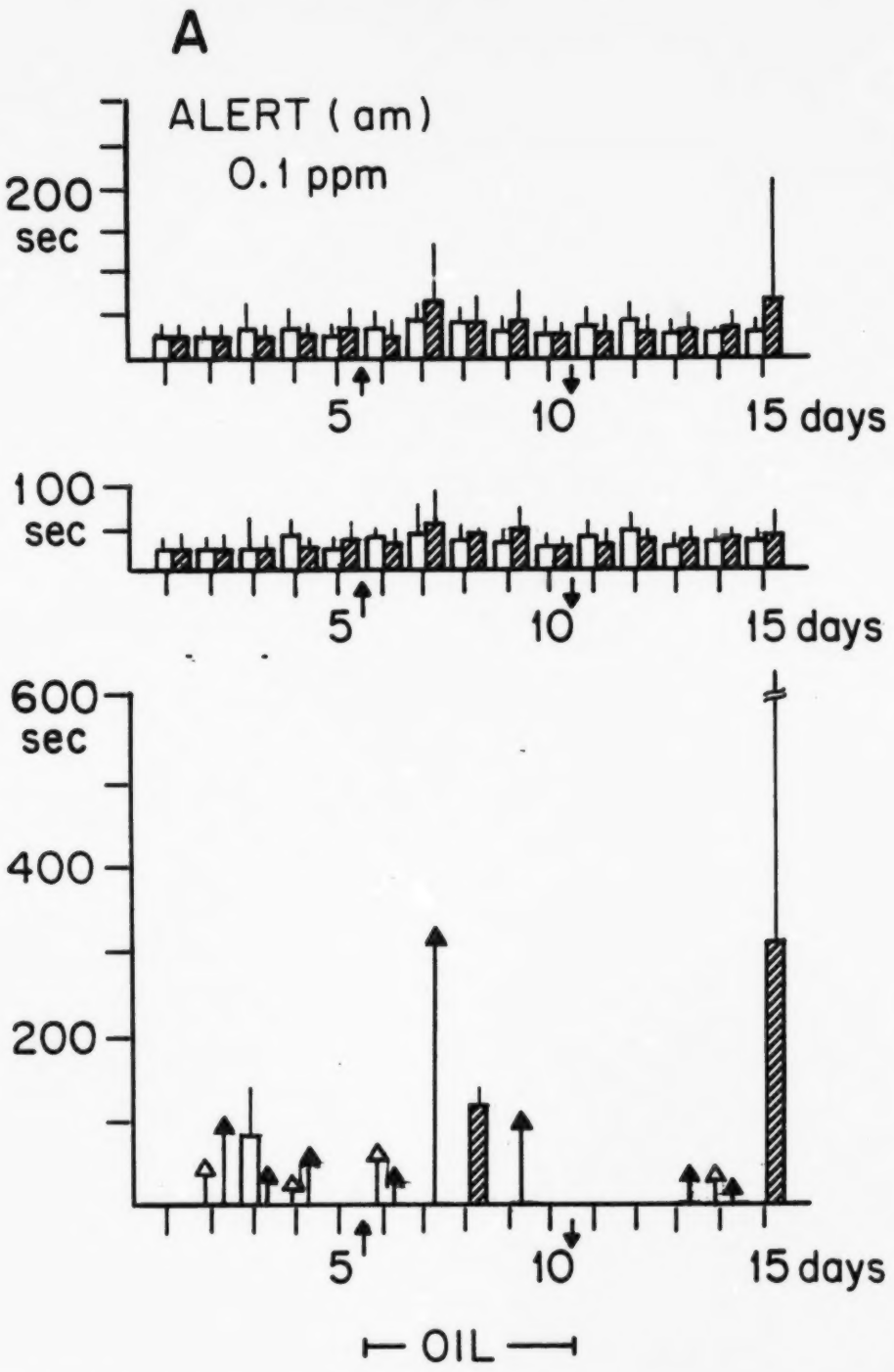
LOW: experiments 1, 2, 3 at 0.1 ppm oil exposure. HIGH: experiments 4, 5, 6 at 0.3 ppm oil exposure. NO ANT: experiment 8 at 0.3 ppm oil exposure with lobsters without antennules. For all experiments, date and mean temperature are indicated. Cont: control animals. Oil: experimental animals. PRE:OIL refers to the statistical comparison of the 5 PRE exposure control days with the 5 OIL exposure days; in PRE:POST the same 5 control days are compared with the 5 POST exposure recovery days.

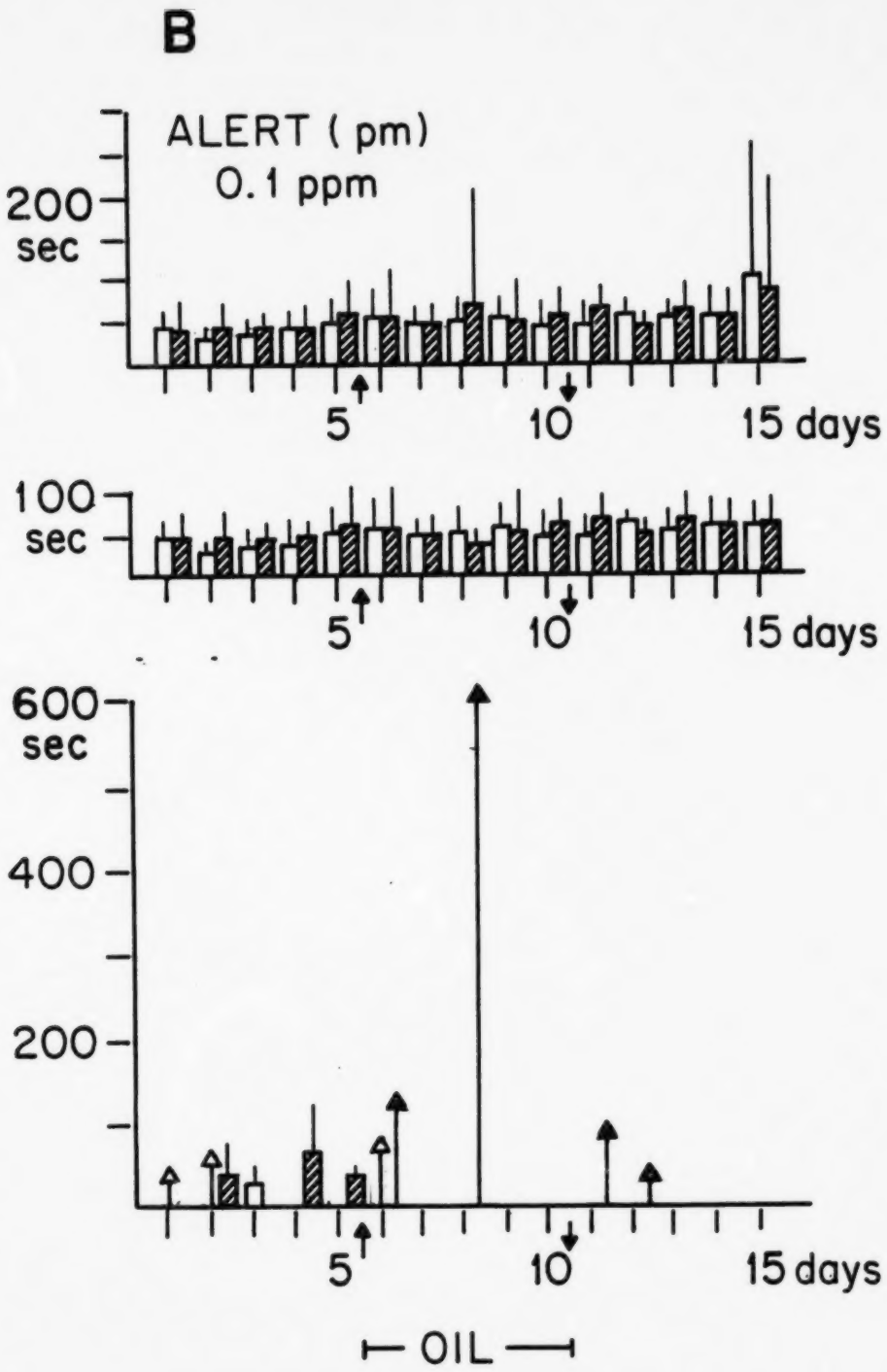
individuals more equally, or at least would have caused similar effects in a given individual on all days of exposure. This is further demonstrated when examining whether animals which fed normally (HIT) on a particular day also alerted and searched normally on that day. Alert and search are clearly under (chemo)sensory control. For this analysis the three higher exposure experiments (#4, 5, 6) were pooled, and for each day all the experimental and control lobsters were divided into two categories: those that found their food in 10 minutes (HIT) and those that did not. The day-by-day ALERT and WAIT times of the feeding and the non-feeding categories were then compared between experimental and control groups. As expected from Fig. 5, animals in low exposure experiments showed no effects.

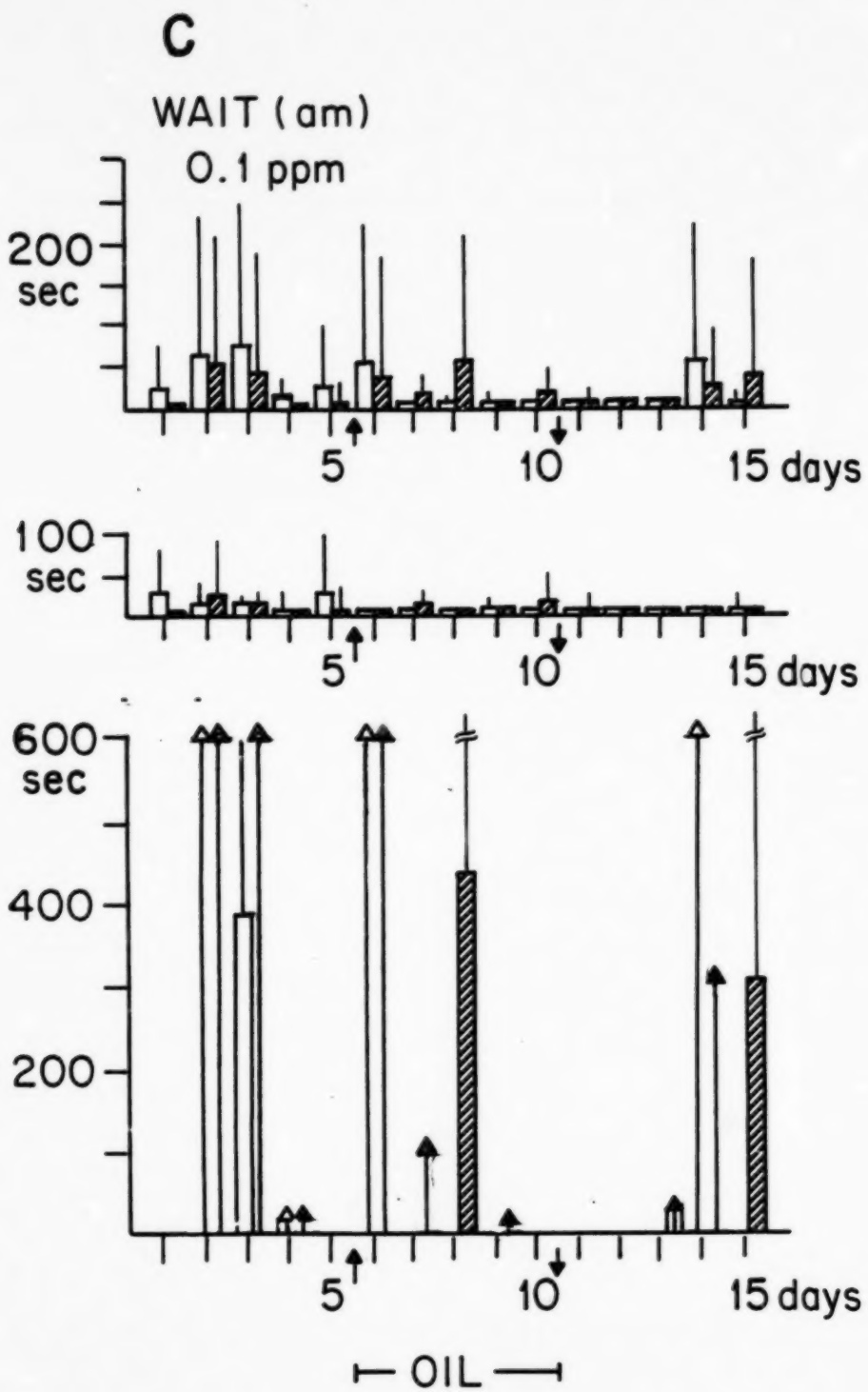
The results of the above analysis are presented in Fig. 8, A-H. Morning and afternoon observations are summarized separately, since differences between AM and PM feeding may show motivational factors (i.e. hunger level, circadian rhythms, light exposure). The top portion of each figure shows the daily mean ALERT or WAIT time in seconds of all animals, comparing control and experimental groups, regardless of whether they reached their food (HIT). The middle graph shows mean times for only those animals which HIT, while the bottom graph shows mean times for only those that did not HIT within 10 minutes. Fig. 8 shows that all animals which HIT also had normal ALERT and WAIT times; i.e. there are no oil effects in 0.1 and 0.3 ppm exposed animals which go out and HIT within 10 minutes. However, there is a difference in 0.1 and 0.3 ppm exposures for animals that do not HIT. The low exposures caused no or perhaps only a slight delay in ALERT and WAIT for these animals, but the higher exposures caused a delay during PM only (Figs. 8F, H), with a great increase in ALERT and WAIT times during OIL and POST periods (the last 10 days of each experiment). When "single animal" points (triangles) are excluded due to delays which occurred in control and experimental groups alike, the results are even more clear cut! In AM recordings (Figs. 8E, G) the effects of oil are less evident, since delays also occur in the PRE control period. Occasionally animals from the control group are also involved in no HIT and long delays in ALERT and WAIT. Response differences between AM and PM observation periods, and the fact that the WAIT phase is affected, suggest a motivational effect (see discussion). The motivation hypothesis is also supported by the fact that the longer ALERT and WAIT times occurred in different animals on different days.

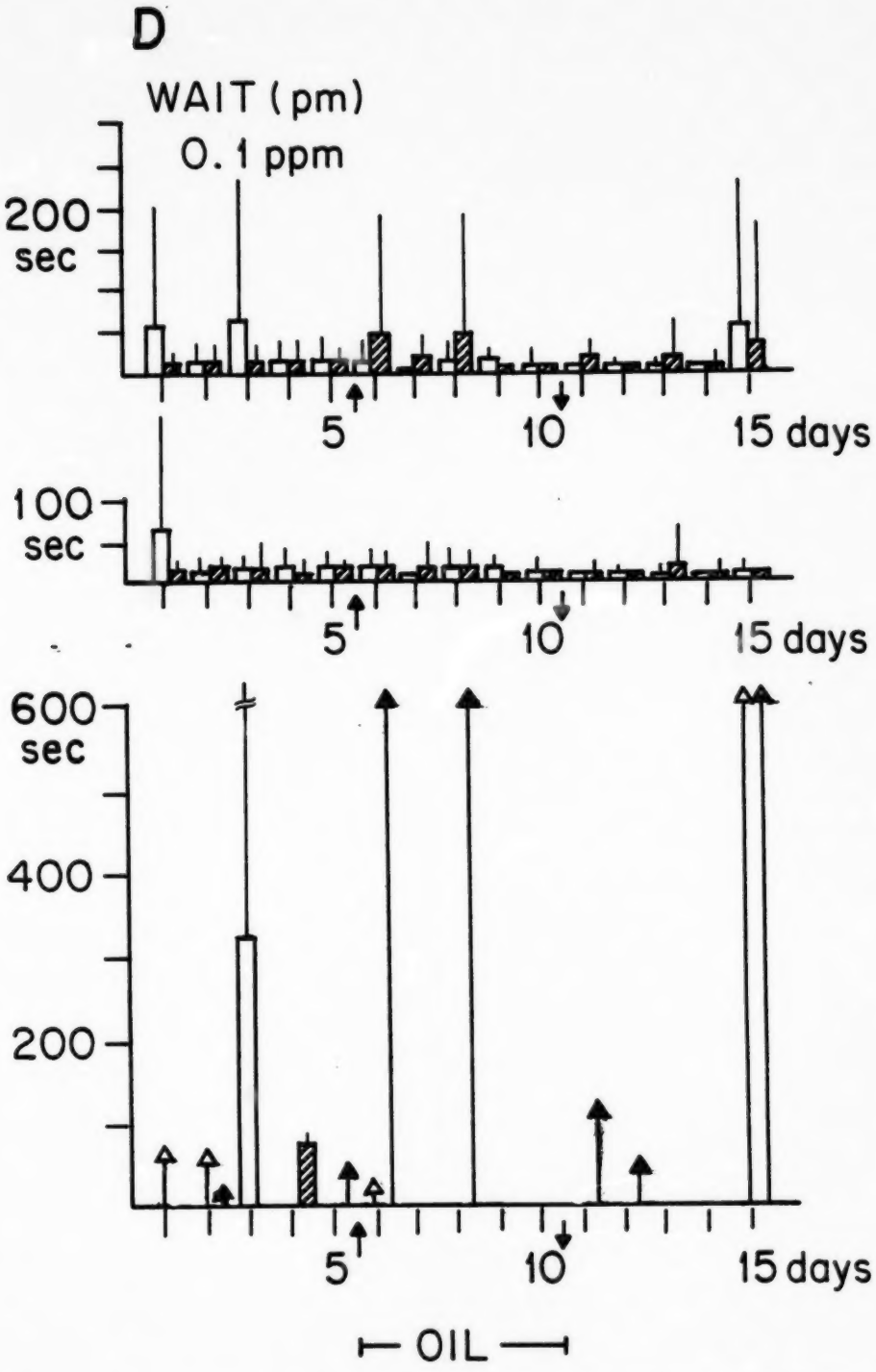
Oil Effects on Behavior Units

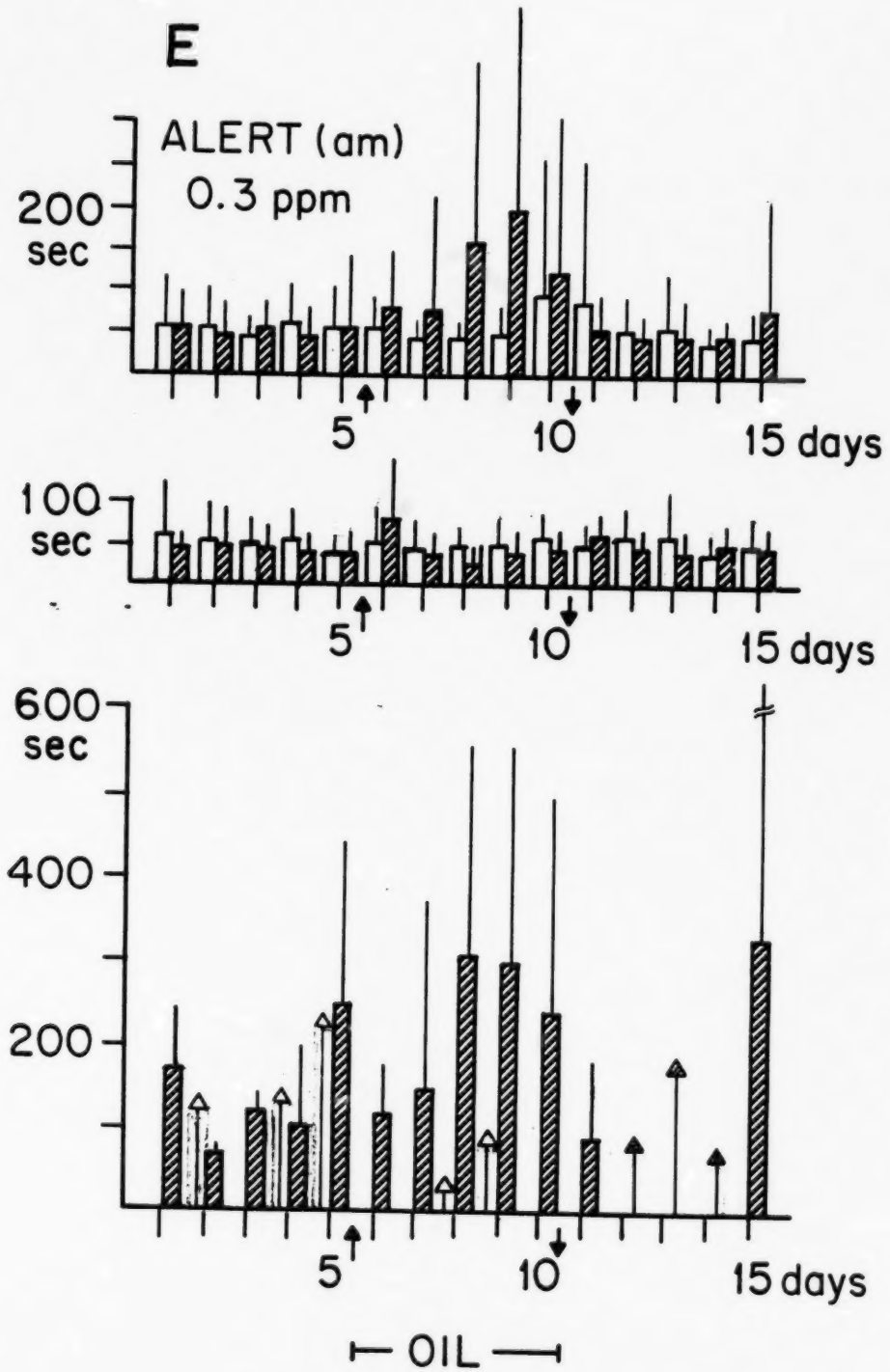
To measure possible effects of oil exposure on the observed behavior units, the frequency of these units was compared for PRE, OIL and POST periods. Out of about 50 behavior units recorded (Table 2), only 4 showed consistent changes in all individual lobsters, allowing overall statistical differences to appear. In all other behavior units, individual animal differences were greater than differences in experimental treatments. Affected units are: "rear", "hesitate", "pounce" and "fake feed". The results were calculated separately for AM, PM and AM + PM, and compared between control and experimental groups (Table 4). Very few values (8 out of 105 calculated) turned out to be significantly different. Those differences which were significant did occur in four different experiments, supporting their reality. "Rearing" frequencies decreased with oil exposure, such as found in

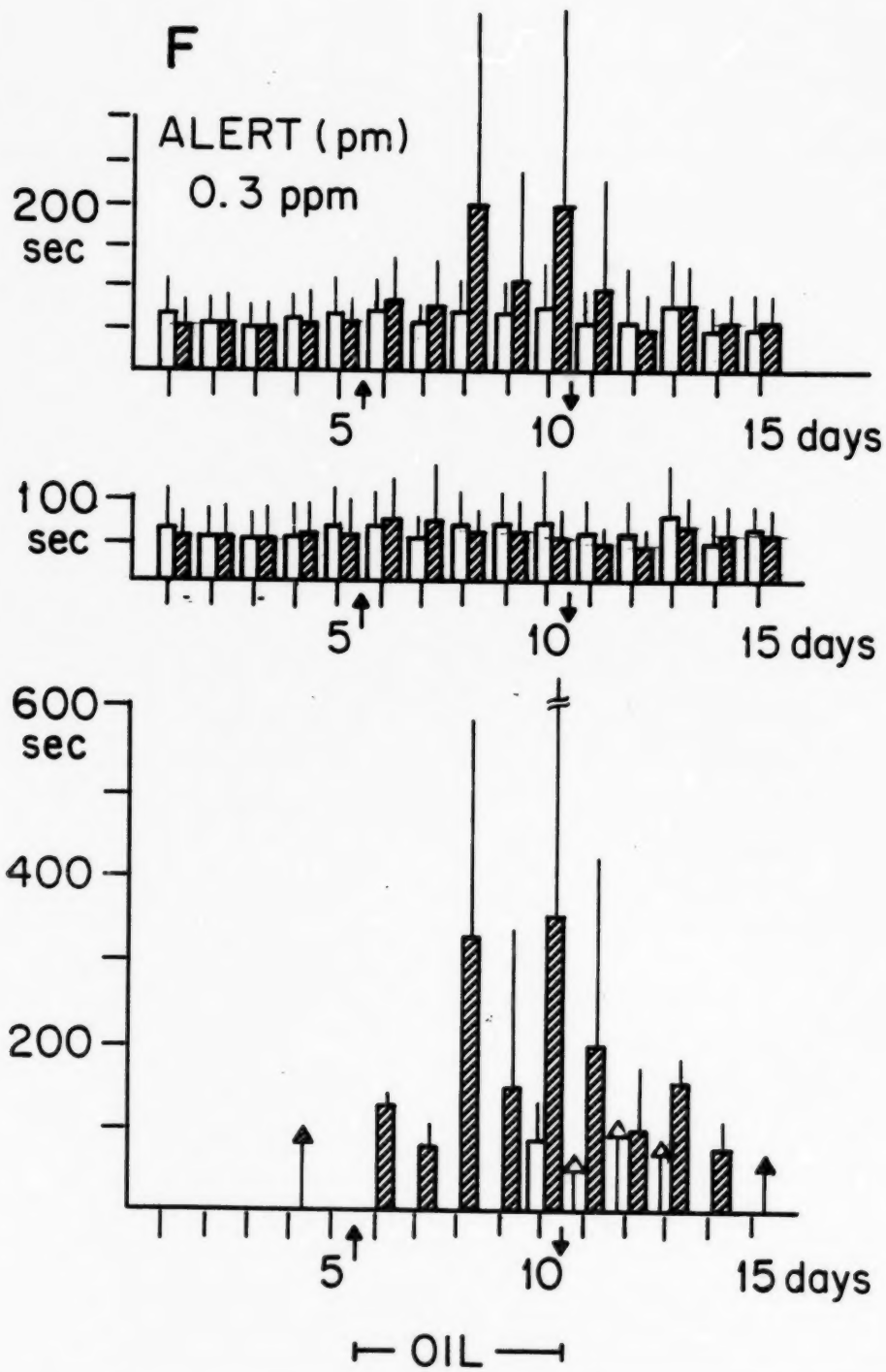

A
ALERT (am)
0.1 ppm
200
sec
5
10
15 days
100
sec
5
10
15 days
600
sec
400
200
5
10
15 days
OIL

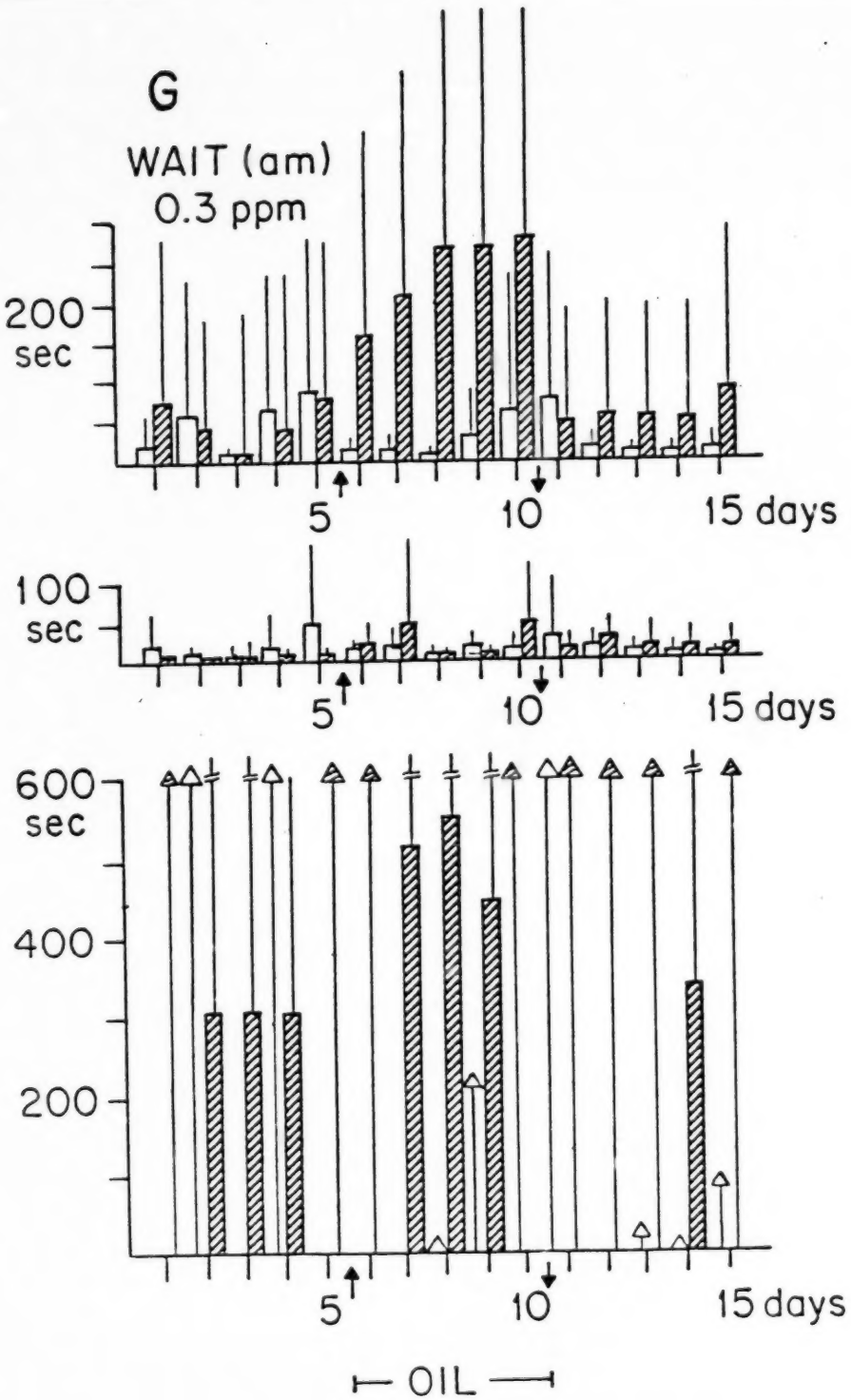

B
ALERT (pm)
0.1 ppm
200
sec
5
10
15 days
100
sec
5
10
15 days
600
sec
400
200
5
10
15 days
OIL


C
WAIT (am)
0.1 ppm
200
sec
5
10
15 days
100
sec
5
10
15 days
600
sec
400
200
5
10
15 days
OIL


D
WAIT (pm)
0.1 ppm
200
sec
5
10
15 days
100
sec
5
10
15 days
600
sec
400
200
5
10
15 days
OIL


E
ALERT (am)
0.3 ppm
200
sec
5
10
15 days
100
sec
5
10
15 days
600
sec
400
200
5
10
15 days
OIL


F
ALERT (pm)
0.3 ppm
200
sec
5
10
15 days
100
sec
5
10
15 days
600
sec
400
200
5
10
15 days
OIL


G
WAIT (am)
0.3 ppm
200
sec
5
10
15 days
100
sec
5
10
15 days
600
sec
400
200
5
10
15 days
OIL

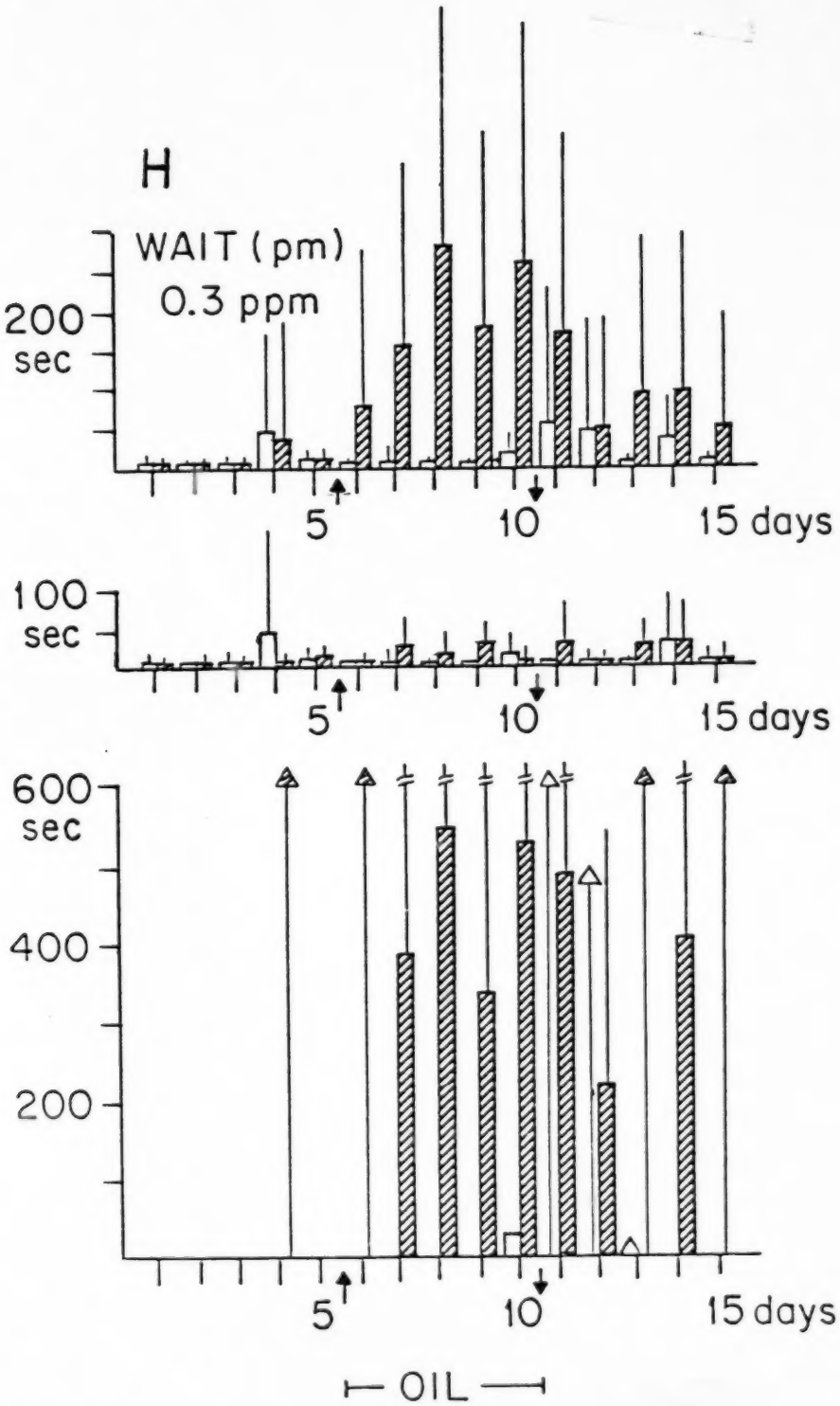

H
WAIT (pm)
0.3 ppm
200
sec
5
10
15 days
100
sec
5
10
15 days
600
sec
400
200
5
10
15 days
OIL

TABLE 4. SUMMARY OF NONPARAMETRIC MULTIPLE COMPARISONS OF RANKED EXPOSURE EFFECTS ON THE FREQUENCY OF 4 BEHAVIOR UNITS *

Unit	Experiment Number	Test	Result	P	Conclusion
Rear	2(am)	Dunnett's	2.887	$p < .01$	Pre > Oil
	2(am + pm)	Dunnett's	3.03	$p < .01$	Pre > Oil
	8(pm)	Dunnett's	2.742	$p < .01$	Pre > Oil
Hesitate	1(am + pm)	Dunnett's	2.312	$.01 < p < .05$	Oil > Pre
	6(am + pm)	Dunnett's	2.21	$.01 < p < .05$	Post > Pre
Pounce	8(pm)	SNK	3.470	$.01 < p < .025$	Post > Oil
	8(am + pm)	SNK	3.471	$.01 < p < .025$	Post > Oil
Fake Feed	6(pm)	Dunnett's	2.454	$p < .01$	Oil > Pre

* Dunnett's: Dunnett's Test (Szar, 1974)
SNK: Student-Newman-Keuls Multiple Range Test (Szar, 1974)
The conclusion column indicates in which of the three 5-day periods of the experiment the frequency of the particular behavior unit was significantly greater.

experiment 2 (AM) and (AM + PM) and in experiment 8 (PM). "Hesitate" frequencies increased with oil exposure; this was found in experiment 1 (AM + PM) and experiment 6 (AM + PM). "Pounce" frequencies increased during the POST recovery period in experiment 8 (PM) and (AM + PM). "Fake feed" was more frequent during OIL in experiment 6. One other unit ("Return") was consistently present, but never significantly affected by oil.

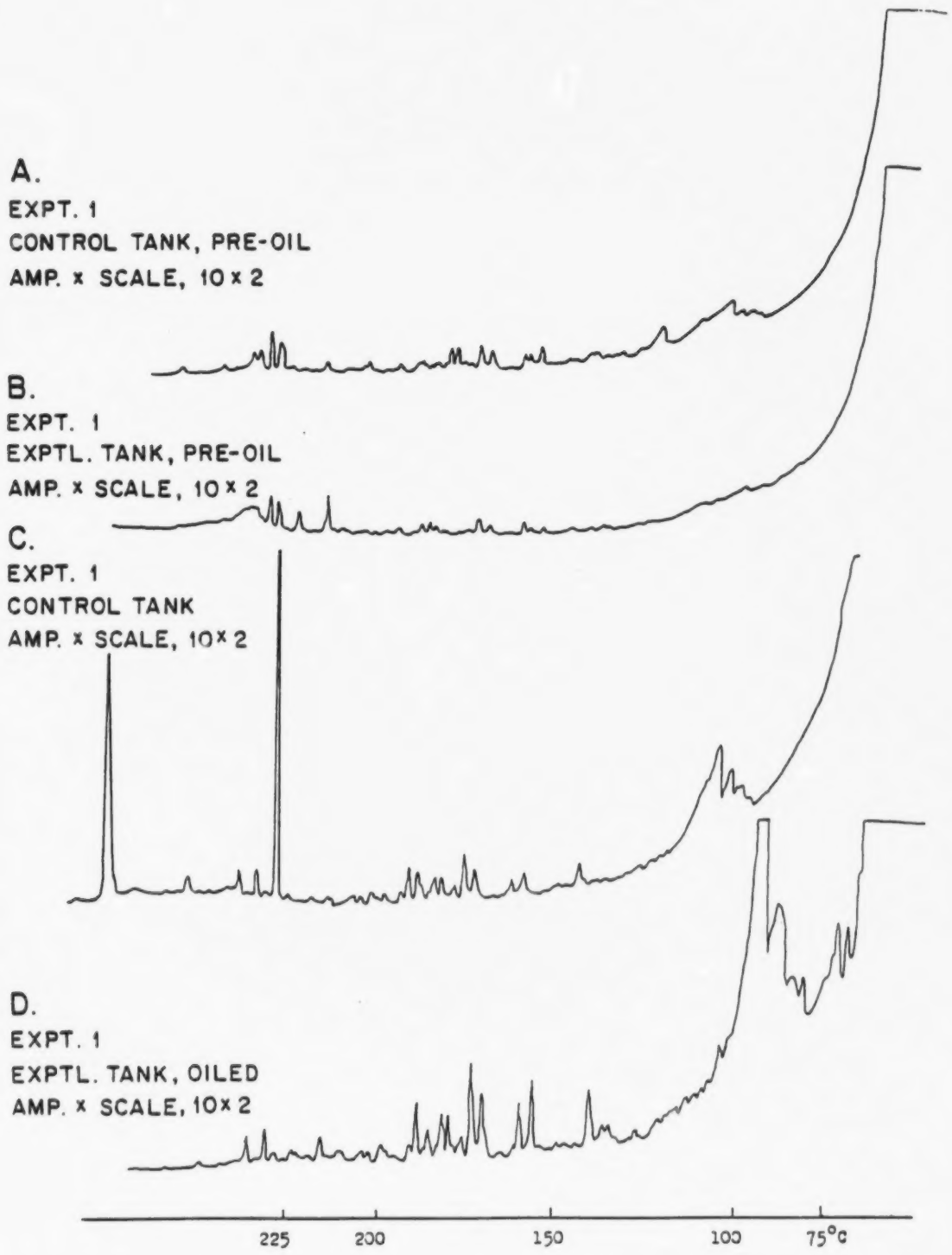
CHEMISTRY

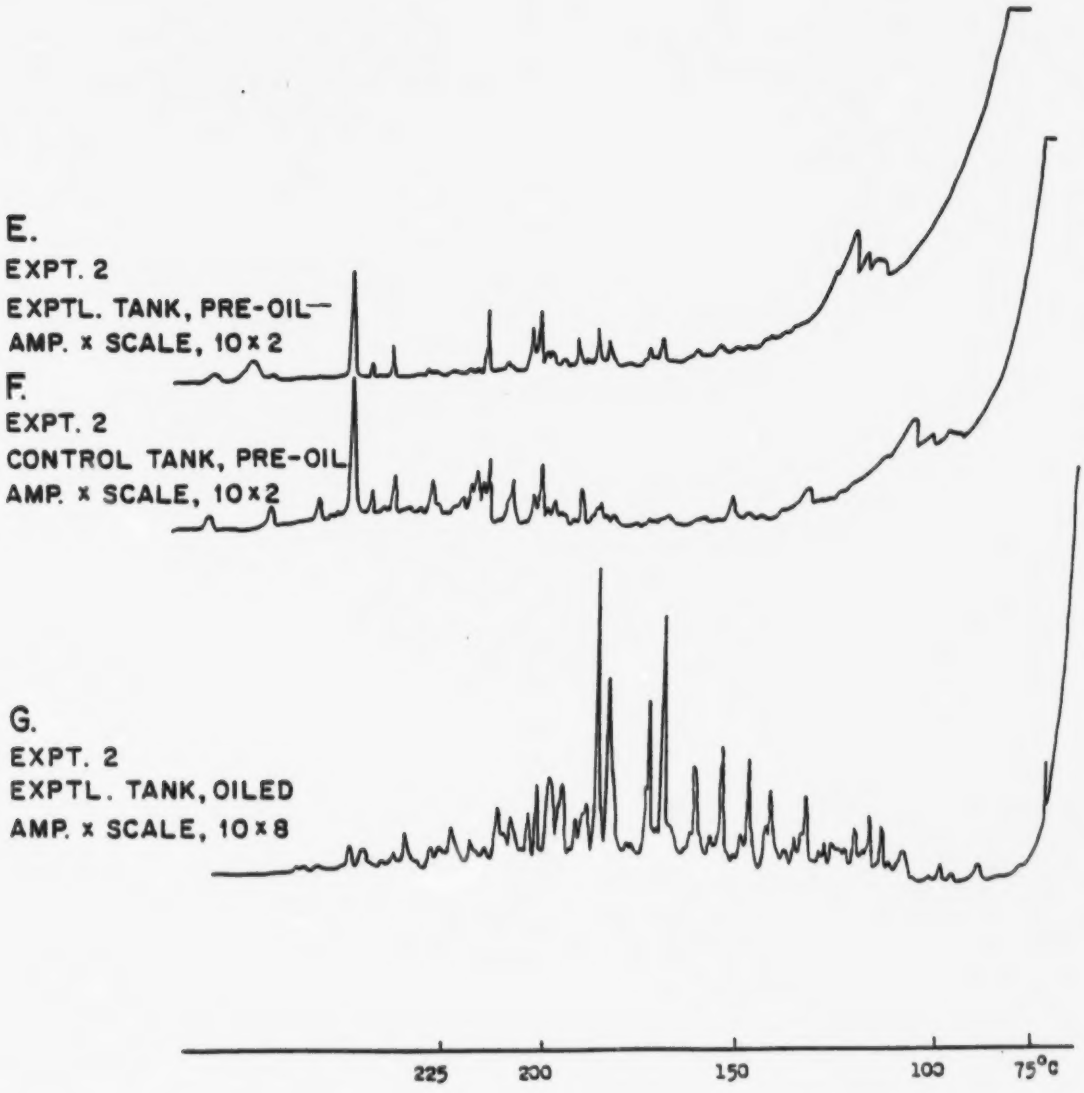
Water quality measurements for all experiments showed salinity and pH remained constant, ammonia remained undetectably low, and O_2 remained at saturation. Ambient water temperature fluctuated with season and is listed for each experiment in Table 1 and Fig. 6.

Hydrocarbon concentrations (± SEM) determined by infrared spectroscopy (IR) are shown in Figure 6. Background hydrocarbon levels were 0.03-0.08 ppm. In one experiment (#4) both control and experimental tanks had unusually high hydrocarbon backgrounds (IR) before oil was added (Fig. 6D). This background is attributable either to hydrocarbons of planktonic origins or to petroleum contamination of the laboratory's water supply by harbor traffic. Gas chromatographic analysis of water from these control tanks shows that petroleum contamination is less likely. Biological results from experiment 4 support that conclusion (Figs. 6D, 7); see also below.

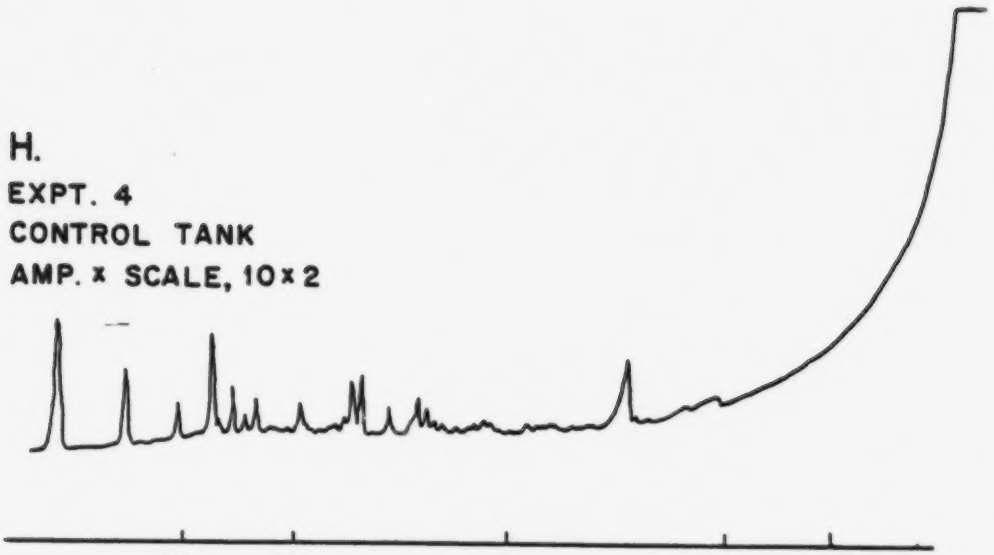
Using as criteria 1) presence of an "unresolved complex mixture" (UCM) (Boylan and Tripp, 1971) and 2) presence of a series of peaks representing n-alkanes, GC analysis indicates that during OIL periods petroleum hydrocarbons were present in the experimental tanks (Figs. 9d, 9g, 9i, 9m, 9o, 9q and 9s). Petroleum hydrocarbons continued to be detected in an experimental tank for up to 12 hrs after oil injection was stopped (Fig. 9m). In low (0.1 ppm) oil exposure experiments the pattern of petroleum peaks was not readily discernible from background (Fig. 9d).

Samples from control tanks were run at a higher instrument sensitivity. Even then, neither UCM's nor n-alkane series were observed, indicating the absence of petroleum in control tanks (Fig. 9). Instead, several other patterns of peaks are noted which may account for much of the IR detectable background in control and PRE experimental tanks. Early eluting polar compounds are found in most samples run at high sensitivity (Figs. 9a, 9c, 9d, 9e, 9f, 9j, 9l, 9n, 9p) and account for a large fraction of total peak area. These compounds are barely detectable in the experimental sample traces. Another sizeable fraction of the total peak area in control samples is attributable to compounds that eluted after most of the petroleum hydrocarbons (after column temperatures of 200°-250° C). While the identity of compounds found in control tank water is unknown, the distribution of peaks indicates that lobsters were not exposed to petroleum hydrocarbons in the control tanks or during the PRE period in experimental tanks. These recovered hydrocarbons are probably of recent biological origin.

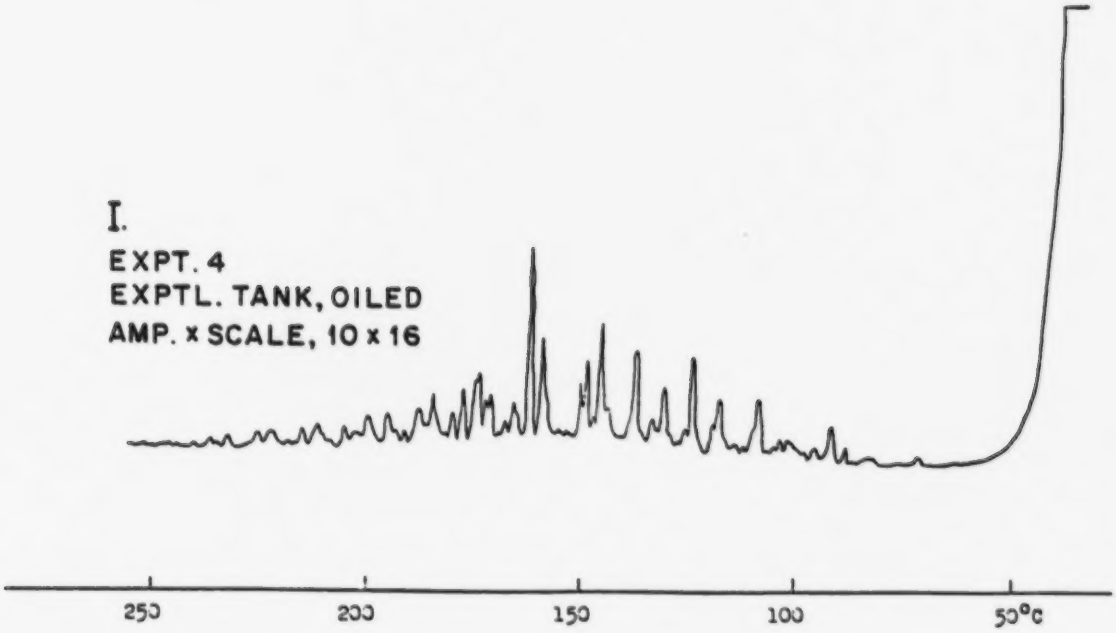

A.
EXPT. 1
CONTROL TANK, PRE-OIL
AMP. x SCALE, 10 x 2
B.
EXPT. 1
EXPTL. TANK, PRE-OIL
AMP. x SCALE, 10 x 2
C.
EXPT. 1
CONTROL TANK
AMP. x SCALE, 10 x 2
D.
EXPT. 1
EXPTL. TANK, OILED
AMP. x SCALE, 10 x 2
225
200
150
100
75°C

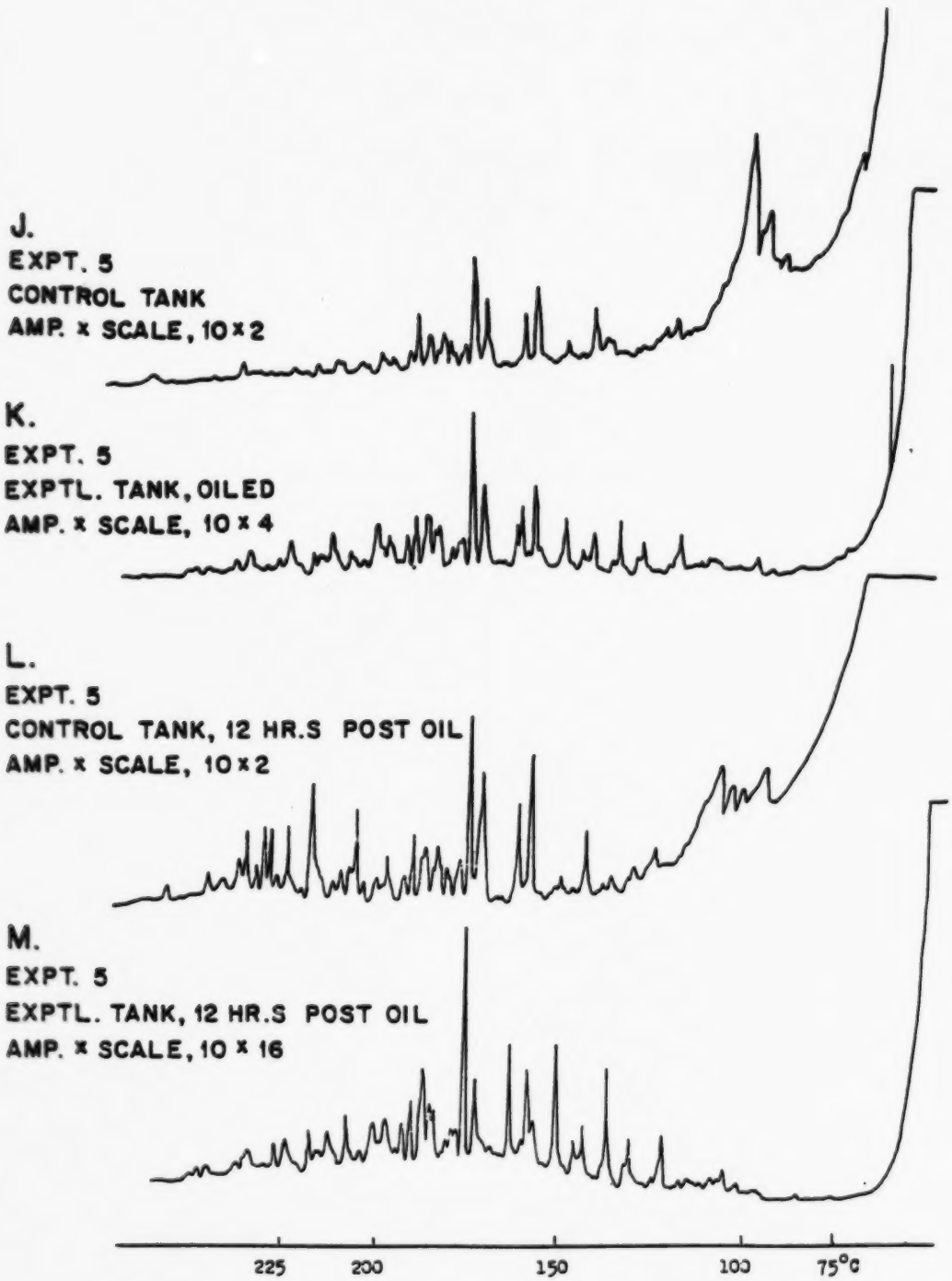

E.
EXPT. 2
EXPTL. TANK, PRE-OIL—
AMP. x SCALE, 10x2
F.
EXPT. 2
CONTROL TANK, PRE-OIL
AMP. x SCALE, 10x2
G.
EXPT. 2
EXPTL. TANK, OILED
AMP. x SCALE, 10x8
225
200
150
100
75°C

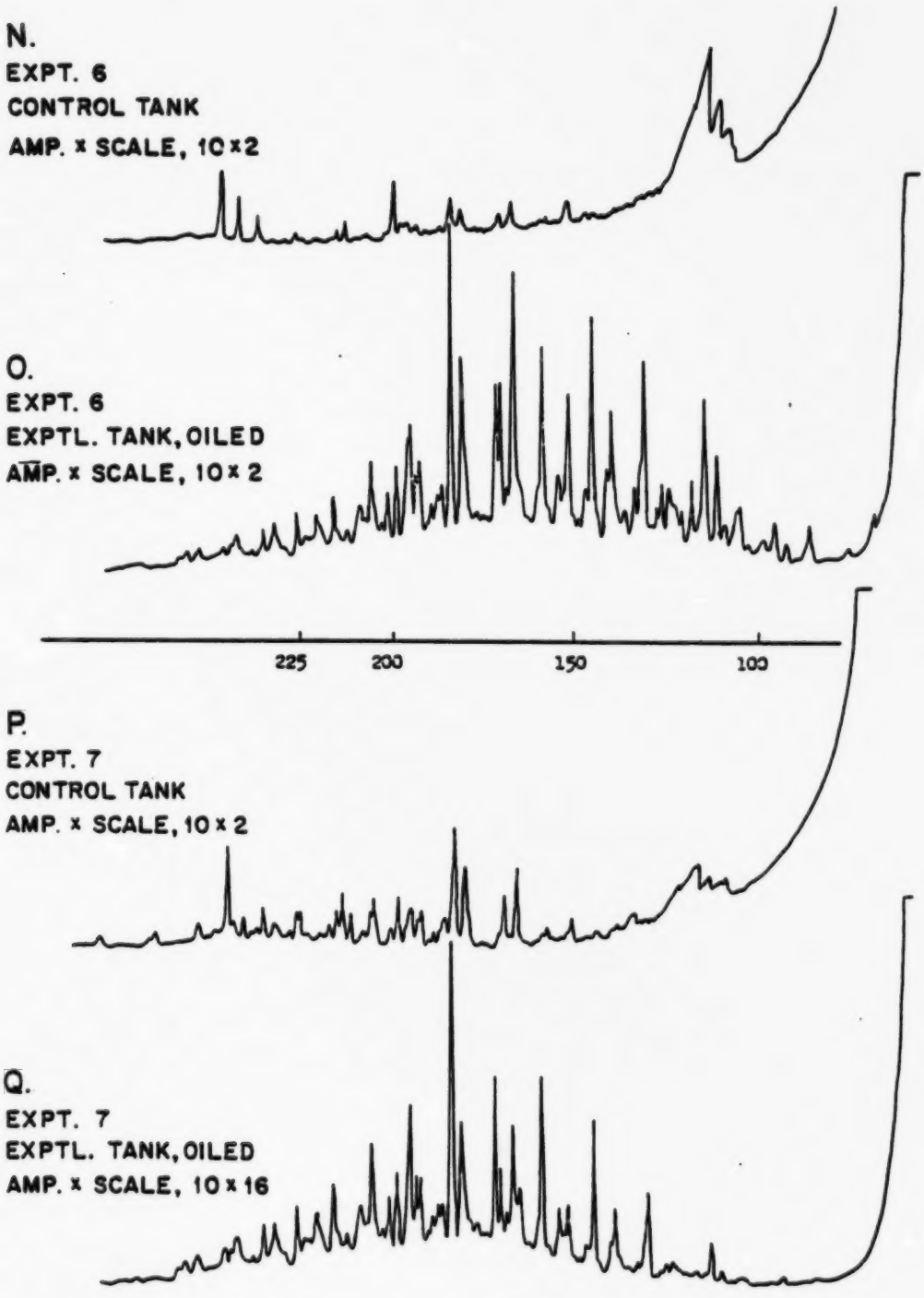
H.
EXPT. 4
CONTROL TANK
AMP. x SCALE, 10 x 2

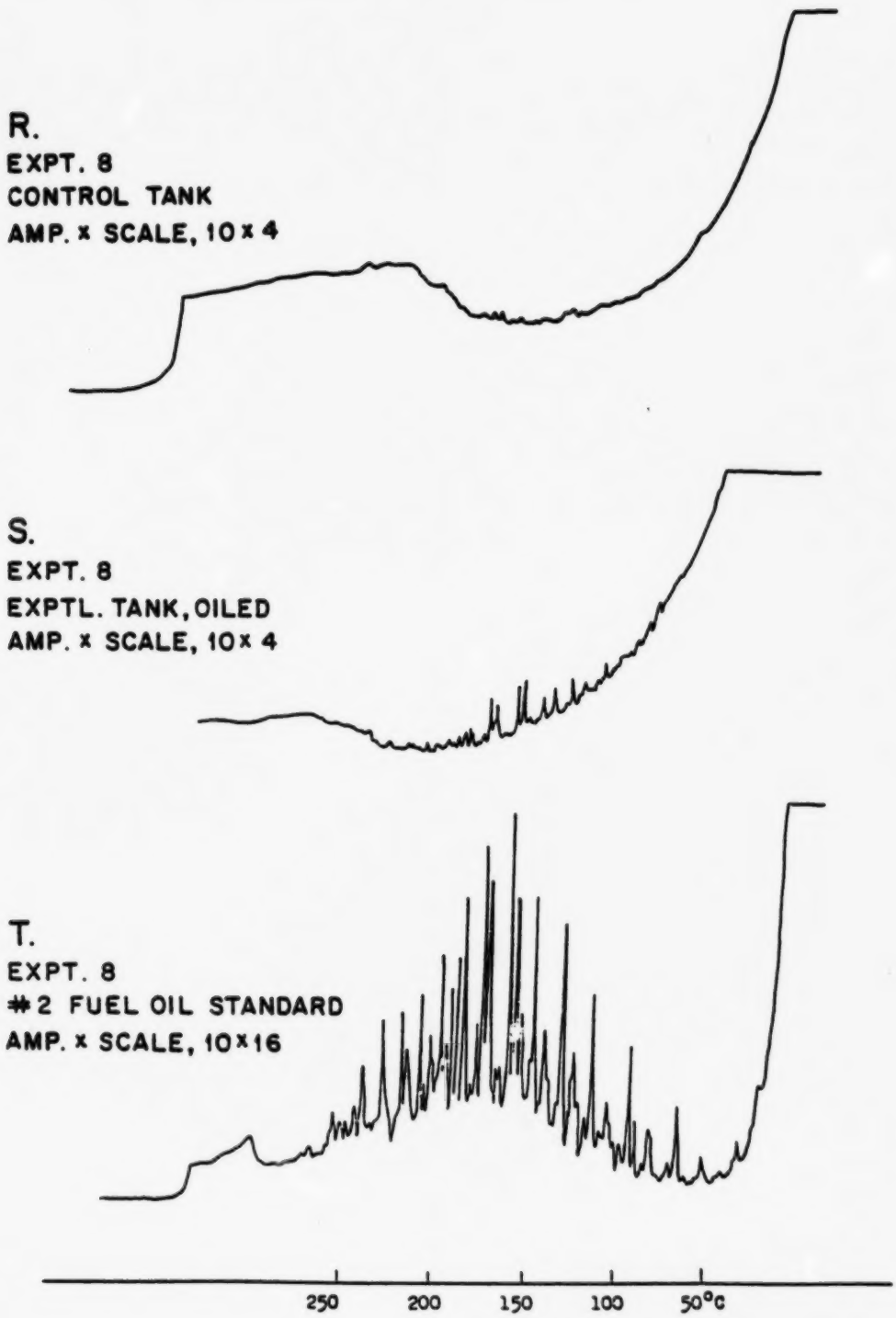


I.
EXPT. 4
EXPTL. TANK, OILED
AMP. x SCALE, 10 x 16




J.
EXPT. 5
CONTROL TANK
AMP. x SCALE, 10x2
K.
EXPT. 5
EXPTL. TANK, OILED
AMP. x SCALE, 10 x 4
L.
EXPT. 5
CONTROL TANK, 12 HR.S POST OIL
AMP. x SCALE, 10x2
M.
EXPT. 5
EXPTL. TANK, 12 HR.S POST OIL
AMP. x SCALE, 10 x 16
225
200
150
100
75°C


N.
EXPT. 6
CONTROL TANK
AMP. x SCALE, 10x2
O.
EXPT. 6
EXPTL. TANK, OILED
AMP. x SCALE, 10x2
225
200
150
100
P.
EXPT. 7
CONTROL TANK
AMP. x SCALE, 10x2
Q.
EXPT. 7
EXPTL. TANK, OILED
AMP. x SCALE, 10x16


R.
EXPT. 8
CONTROL TANK
AMP. x SCALE, 10 x 4
S.
EXPT. 8
EXPTL. TANK, OILED
AMP. x SCALE, 10 x 4
T.
EXPT. 8
#2 FUEL OIL STANDARD
AMP. x SCALE, 10 x 16
250
200
150
100
50°C

NEUROPHYSIOLOGY

Lobsters from three different experiments (#5, 7, 9) were used to determine the effects of prior oil exposure levels on chemoreceptor responses to food odors and oil stimuli. The numerical results are listed in Table 5. A total of 116 antennules was used; about half of these were controls. All antennules together provided 784 live nerve bundles, of which 423 were chemoreceptive. Thus, on average 7 spontaneously active nerve twigs per antennule were used; see "Total" column in Table 5a. Of these just over half were responsive to food odor stimuli. The proportions of chemoreceptive units per active nerves per antennule were similar for all treatment groups, indicating no effect of treatment on receptor viability. The apparent exception of POST 1.5 ppm treatment is based on only 4 antennules, which yielded proportionally higher numbers of active nerves (43 out of 4) and chemoreceptive units (30 out of 43) (Table 5a).

The three effects of oil (sensing oil, interference of oil with food odor stimuli, and abnormal bursting) are shown in Fig. 10. These examples show that both in control and experimental animals (1) No. 2 fuel oil (WAF) itself is a chemical stimulus for primary receptor cells, (2) that the presence of oil in the seawater background can change the response pattern of the small nerve bundle to mussel juice food stimulus, and (3) the occurrence of abnormal bursting patterns. The frequency with which these response types occurred in the different treatment groups are listed in Table 5b. On average ("Total") 13% of the chemoreceptive units responded to oil (1 or 3 ppm WAF) as a stimulus. This percentage was lower in controls and 1.5 ppm treatments (0-9%) and higher in 0.3 ppm exposures (19% during OIL and 25% during POST). These differences are perhaps insignificant and no explanation can be offered at this time.

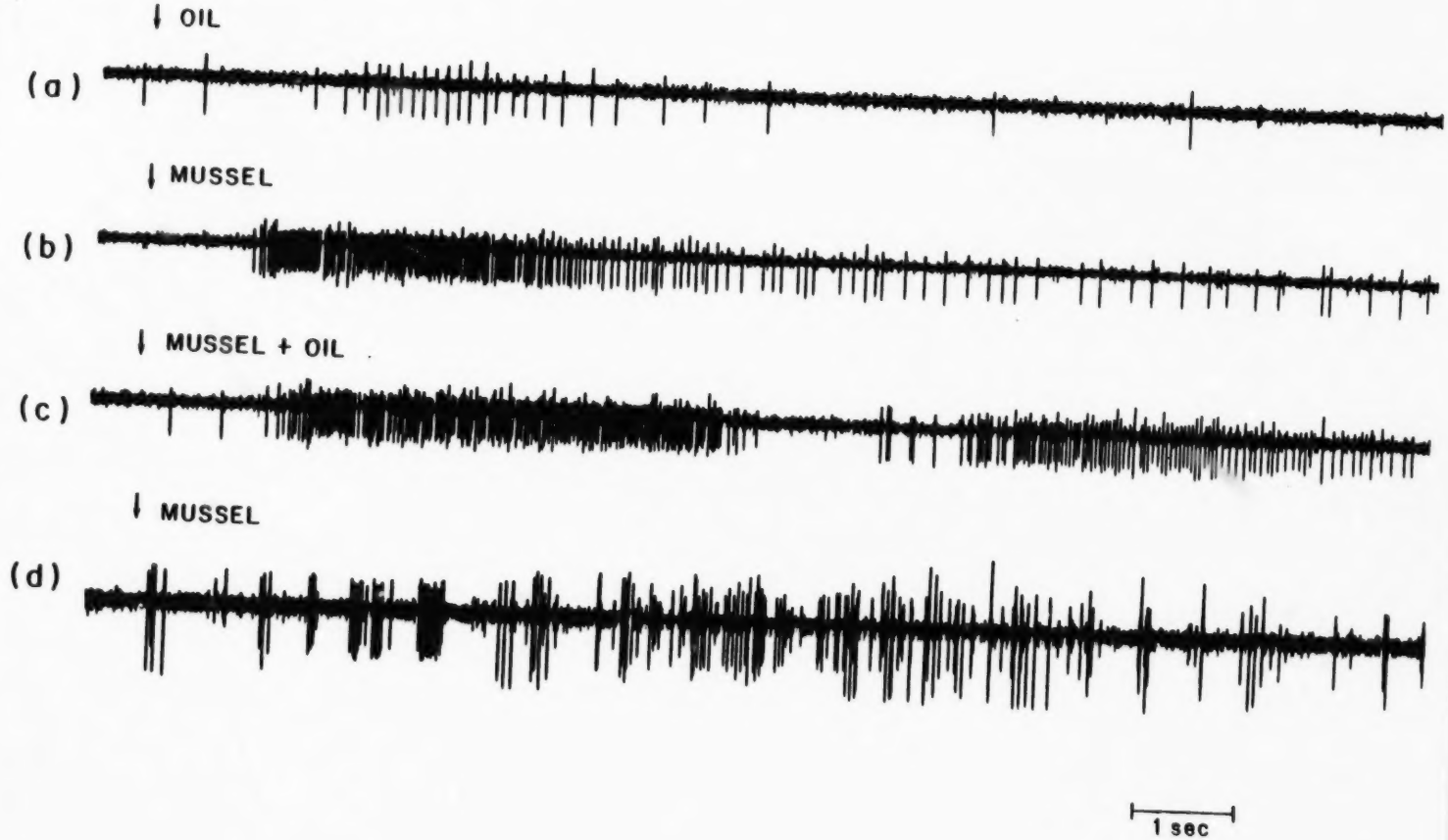
On average 14% of the chemoreceptive units showed oil interference by changing their normal food odor response when oil (1 or 3 ppm WAF) was added. During POST 0.3 ppm and OIL 1.5 ppm treatments this proportion was somewhat greater (21% and 18% respectively), whereas during POST 1.5 ppm none of the units showed this effect. Abnormal bursting was seen too infrequently (less than 2% of the units, total) to attempt quantification. Of these results, the total lack of oil effects on POST 1.5 ppm WAF chemoreceptor nerves is remarkable, particularly since a high proportion of active nerves was normally receptive to food odor.

Partly due to inter-individual differences, which were greater than treatment differences, oil exposed and control animals did not show significant differences in their chemoreceptor responses to oil or food odor. Thus, the attempt was abandoned to correlate gravity of behavioral disturbance of individual animals in experiment 7 to the degree of their chemoreceptor abnormality. Moreover, the behavioral results were unavoidably confounded by effects of cutting antennules during the experiment, so that no useful comparisons can be made.

TABLE 5. NEUROPHYSIOLOGICAL RESPONSES OF ANTENNULAR CHEMORECEPTORS OF LOBSTERS IN DIFFERENT TREATMENT GROUPS.

	Control	0.3 ppm (from expts #5,7) OIL	0.3 ppm (from expts #5,7) POST	1.5 ppm (from Exp. #9) OIL	1.5 ppm (from Exp. #9) POST	Total
A) Number of Tests						
Antennules	52	38	8	14	4	116
Active Nerves	320	315	59	97	43	784
Chemoreceptive Units	158	163	28	44	30	423
B) Response Types						
Oil Sensing	15(9)*	31(19)	7(25)	4(9)	0	57(13)
Oil Interference	21(13)	23(14)	6(21)	8(18)	0	58(14)
Abnormal Bursting	1	3	1	3	0	7

* Numbers in parentheses: the percentage of the chemoreceptive units in the treatment group showing oil sensing and oil interference respectively.


OIL
(a)
MUSSEL
(b)
MUSSEL + OIL
(c)
MUSSEL
(d)
1 sec

SECTION 6

DISCUSSION

The results from the chemical analyses of lobster tank water, IR and GC data combined, show that experimental tanks received petroleum (Exxon No. 2 fuel oil WAF) at the approximate rates intended, and that most petroleum cleared out quickly but not completely until 12-48 hrs after oil inflow had stopped. It is also shown that neither control tanks nor experimental tanks PRE-oil carried significant amounts of petroleum. The IR-observed background of about 0.05 ppm consisted mostly of non-petroleum lipids. Behavioral results did not show observable differences between experiments with fluctuating vs. level petroleum dosing. Other water quality criteria, pH, oxygen, ammonia and salinity remained at satisfactory levels, as one might expect in a rapid flow-through, ambient system. Based on these results, the experimental conditions are considered acceptable for data interpretation.

The behavioral experiments fall into three groups, which together bracket the sublethal levels of exposure. The 0.1 ppm level caused hardly any observable effects. Possibly the decrease in REAR behavior, the increase in HESITATE behavior and the longer WAIT phase (Table 4 and Fig. 8) might be attributed to petroleum at this LOW level. On the other hand, the 1.5 ppm level constitutes a HIGHER sublethal concentration, where gross neuromuscular defects appear within a few hours and last for several days afterwards, even after only 30 hrs of exposure (Figs. 1 and 6J). Thus, within the relatively narrow range of 0.1 to 1.0 ppm, No. 2 fuel oil (WAF) poses a sublethal stress on feeding in subadult and early adult lobsters.

Experiments 4-8, excluding #7 for confounding effects of antennule cutting, were done at the HIGH exposure level of 0.3 (0.24-0.5) ppm. In all four experiments similar effects were observed which resulted in greatly decreased feeding behavior. Whether one considers the final criterion of finding food after searching (Fig. 6), or the more detailed description of completion of the entire feeding behavior sequence (Fig. 7), or statistically significant differences in duration of phases before and during exposure (Table 3), the overall results are the same: lobsters exposed for five days to 0.3 ppm No. 2 fuel oil WAF are less likely to look for food and eat.

This answers the first of our questions: We documented a clear sublethal exposure dose which affects a number of quantifiable behavioral parameters. Most effective were the measurements of feeding behavior phases (occurrence and duration). In contrast, great individual differences in lobster behavior made the analysis of behavior unit frequencies a poorer index of oil stress, although this is a potentially sensitive approach.

The neurophysiological experiments on antennular chemoreceptors showed that 0.1 ppm No. 2 fuel oil WAF is both a chemical stimulus and interferes with the cell's normal response to a common food odor (standard mussel juice). There was furthermore no clear difference in chemoreceptor responses between control and exposed lobsters. The latter means that inferference with specifically chemoreception is probably not the (only) cause for failures in feeding behavior due to petroleum exposure. However, from pervious experiments on lobsters (Atema, 1976) we believe that different petroleum fractions and concentrations can act as chemical attractants, repellents, and neurotoxins. Thus, lobsters may be subjected to a mixture of chemical stimuli in the presence of food and petroleum. This may well cause sensory "confusion" and hence inappropriate responses, quite apart from general neurotoxic effects, which may render the animal unmotivated, uncoordinated, and unresponsive.

Lack of coordination and responsiveness was obvious at HIGHER (1.5 ppm) sublethal exposure. Such general, presumably neurotoxic, effects may appear as lack of motivation in the middle of the sublethal range (0.3 ppm). This possibility has been explored in the analysis presented in Fig. 8, from which it appears that animals that do leave their shelter, and search and eat, do so with normal alert and wait times, regardless of the 0.3 ppm oil exposure. Thus, the effect of 0.3 ppm oil exposure is that far more animals more frequently refuse to leave their shelters, or even show a visible alert to the food odor. This argues against purely (chemo)sensory effects, and for effects on motivational state. These effects are seen in AM, not in PM observations, which is also an indication that petroleum affects hunger motivation. Hence, sensory deficits may not be the primary cause of oil induced behavioral changes. Finally, the feeding behavior of animals without antennules (experiment 8) are equally affected by sublethal petroleum exposure. If the antennular chemoreceptors had been an important mediator in the observed behavioral deficits, one would expect animals without antennules to be less affected by oil. It should be noted that lobsters adjust to finding food without antennules in a few weeks, which is the time between cutting antennules and the beginning of experiment 8. One should also keep in mind that petroleum may affect all chemoreceptors not just antennular chemoreceptors. These other receptors could still mediate oil effects in antennule-ablated animals. However, one can not eliminate these other chemoreceptors without affecting feeding behavior per se (Derby and Atema, 1981; Devine and Atema, 1981).

Either decreased feeding motivation or sensory deficits can be observed in the analysis of behavioral unit frequencies (Table 4). An increase in "Hesitate" and "Fake Feed" means that the animal has trouble locating the food: it stops frequently during its search and attempts feeding on the pebble substrate. A decrease in "Rear" means that the lobster less frequently reaches for the water surface, which normally may perhaps express feeding excitement. Thus, a decrease in "Rear" may be a protective strategy of a wary animal, or a lack of feeding motivation. Since significant changes in unit frequencies occurred in only a few experiments, one should not place too much weight on the results from these analyses.

Hunger is a motivational state we know little about in lobsters; we only observe its effects in their readiness to eat. When we describe feeding

behavior as a sequence of phases (alert, wait, search, hit, eat), each with its own set of sensory inputs and acceptance thresholds (Derby and Atema, 1981; Devine, 1981), we see that exposure to 0.3 ppm petroleum has the overall effect of phases dropping out starting with "EAT" and finally ending with the situation where lobsters do not even show a visible "ALERT" (Figure 7). Different chemical senses both in catfish (Atema, 1971), insects (Dethier, 1976) and lobsters (Derby and Atema, 1981) control different phases of feeding behavior and each subsequent phase in the feeding sequence has a different threshold. For instance, hungry catfish or lobsters may be attracted to and even pick up a piece of foul meat; but they often reject it once they "taste" it in their mouths. Less hungry animals reject at earlier phases of the sequence. Accordingly, the observed effects of oil can be interpreted in three ways, which are probably not mutually exclusive. The 0.3 ppm level of No. 2 fuel oil WAF may interfere with the sensory process of chemoreception, or it may present a competing, generally negative chemical stimulus affecting motivation, or it may have a general neurotoxic effect, which includes sensory and motivational processes. The results of the present study support particularly the last interpretation.

In summary, these and other experiments show that oil does affect lobster chemoreception in two ways: oil is a chemical stimulus (neurophysiological results), which can attract or repel lobsters depending on its chemical composition (Atema, 1976) and on the motivational (hunger) state of the animal (Atema and Stein, 1974); oil interferes with chemoreception by causing a change in the neural spike patterns that normally result from food odor (mussel juice) stimulation, but we do not know how the lobster interprets these patterns. However, three observations argue against the interpretation that the particular behavioral deficits observed in this study during and after exposure to 0.3 ppm No. 2 fuel oil WAF were caused by chemosensory deficits. First, different lobsters show different deficits on different days and not necessarily in those behaviors that are most likely under chemosensory control (Fig. 8). Second, control and experimental lobsters show similar unresponsive behavior on certain days; only the number of such unresponsive days increases with oil exposure (Fig. 8). Third, animals without antennules (and hence an important chemoreceptor organ) respond no differently to oil exposure than normal lobsters (Fig. 7). Together these results support an earlier hypothesis, drawn from results of other experiments on lobster feeding behavior (Atema and Stein, 1974), that oil negatively affects the lobster's motivational state for feeding. The physiological basis for the observed lack of motivation is not known, but may be caused by general neurotoxicity of certain fractions of petroleum. These effects appear quickly (within hours) and are obvious at higher exposure levels ($>$ 1 ppm No. 2 fuel oil WAF) when lobsters lose neuromuscular control. Some die in days at this oil exposure. Otherwise, behavioral recovery is dependent on exposure level and takes about 1 day for 0.3 ppm No. 2 fuel oil WAF. At 0.1 ppm exposure, no significant effects were measured. Apparently there is a limited range for sublethal toxicity for this petroleum fraction, which lies between 0.1 and 1 ppm. Clear sublethal effects on feeding behavior are measured consistently at 0.3 ppm. The ecological implications of impaired feeding behavior are a loss of survival fitness in competition with other animals. Longer search times result in extra energy expenditures and increased exposure to predation.

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FIGURE LEGENDS

1. Top: Normal interaction between non-oil contaminated lobsters when first placed in a small (150 l) aquarium. Under these conditions, lobsters display aggressive behavior; they attack each other with thrusts and jabs of their claws and often lock claws in a push-pull test of strength. Bottom: When the same two lobsters are placed together in a small aquarium after exposure to high concentrations of oil (1-10 ppm range), they totally ignore each other just as they ignore food in feeding experiments. In this illustration, two typical "oiled" postures are shown. One lobster lies listlessly flat with stretched claws and limp legs, occasionally twitching its legs or body. The other stands in a spider-like position, tail closely tucked and high on his legs, claws held close to the body, repeatedly attacking imaginary objects or running into walls. Both animals would be defenseless against attack or predation (from Atema, 1977).

2. Relationship of projects: In the complex context of naturalistic and field studies, results from highly controlled toxicity studies on the sublethal interference of petroleum on behavior can be interpreted with some confidence.

3. Flow-through oil dosing system. Lobsters are held individually in aquaria 1-10 for detailed observation of feeding behavior with and without oil exposure. Animals for neurophysiological studies are held in the lower holding tanks. The oil collecting box and oil filter serve to remove oil from the water, which was to be discarded through the drain.

4. Sample data sheet for behavioral recordings. One such sheet was filled out per animal per 5-day period. Asterisk indicates floor of aquarium with shelter in center top; on this floor plan search paths were sketched for each test.

5. "Olfactometer" for neurophysiological recordings. (A) Stimulus injection into (B) sea water flow over antennule. (C) Oxygenated Ringers saline solution from micropipette perfuses inside of antennule and recording bath (D) where small nerve bundles are picked up by platinum hook electrodes (E). Seawater and saline bath are separated by a Sylgard cork, through which the antennule passes.

6. Number of lobsters not "hitting" or "eating" and concentration of hydrocarbon/lipids (IR-determined total CCl_4 solubles) recovered from daily water samples for all experiments. The nine experiments are individually plotted, showing date of test, temperature course over the 15-day period, and dates when GC analyses were done for Control (✱) and Experimental (✱)

tanks. "Oiled" group of lobsters (n = 6) received No. 2 fuel oil, indicated by arrows (↑, on; ↓, off) during the middle period of the experiment. Lipid levels measured for experimental and control tanks are indicated with standard error. Note different protocol of experiment #9. Oil concentration estimated for experiment #6.

7. Presence and absence of phases of feeding behavior for individual lobsters, grouped by general exposure level: LOW or HIGH CONC. Experiment #8 was done with lobsters without antennules. This chart shows for each lobster at each recording session (every day, AM and PM) how many phases of feeding behavior were completed: the more shaded the block, the more of the behavior sequence was absent. The figure illustrates when and how severely different oil exposures affected the control vs. experimental animals and how long into the POST recovery period the effects lasted.

8. The length of Alert and Wait phases of feeding behavior as a function of grasping food (Hit): a possible measurement of feeding motivation. The durations of Alert and Wait phases are calculated from daily AM or PM observations for 0.1 ppm (experiments #1, 2, 3) and 0.3 ppm (#4, 5, 6) exposure groups. The top of each figure shows the mean alert or wait time in seconds, with top half of standard deviation indicated for all animals combined, blank bars for control groups, hatched bars for experimental groups. The middle graph shows alert or wait times for animals that HIT and the bottom graph for those that did not HIT. Observations always ended at 600 sec. When only a single animal formed a "group" it is indicated with a triangle; blank for control, hatched for experimental.

9. Gas chromatograph (GC) records of recovered petroleum (CH_2Cl_2 solubles). Samples were taken on days indicated with asterisks on Figure 6. Most control samples were run at instrument setting 10 x 2 (amp x scale), and hence 8 times more sensitive than experimental (10 x 16).

10. Examples of neurophysiological responses of antennular chemoreceptors to chemical stimuli. a. Control lobster, response to oil stimulus. b. Control lobster, response to mussel juice stimulus. c. Control lobster, response to mussel juice plus oil stimulus. d. Oil-exposed lobster, response to mussel juice stimulus. Note the differences between b and c which were recorded from the same nerve bundle a few minutes apart. Apparently the addition of oil changes the response to mussel juice. Note also the differences between b and d. The irregular bursting patterns seen in d are observed in oil-exposed lobsters, not in control lobsters. While there still is a response to mussel juice, the response pattern appears erratic. It is not known if this erratic pattern results in a different interpretation by the lobster.